REMARKS

Claims 53-56 and 58-76 were pending in the subject application. By this Amendment, applicants have amended claims 53 and 73, cancelled claims 54-57, and 75-76, and added new claims 77-97. Accordingly, claims 53, 58-74 and 77-97 are being examined.

Support for amended claim 53 and new claims 77-97 can be found in the specification as follows:

Support for amended claim 53 can be found in the originally filed specification, for example, at page 5, lines 18-20; page 60, lines 9-16; page 22, lines 26-30; page 23, lines 24-27; page 51, lines 19-23; and page 77, lines 9-27.

Support for new claim 77 can be found in the originally filed specification, for example, at page 61, lines 1-16.

Support for new claim 78 can be found in the originally filed specification at page 60, lines 9-16; page 51, lines 19-23; page 22, lines 26-30; page 23, lines 24-27.

Support for new claim 79 can be found in the originally filed specification at page 60, lines 9-16.

Support for new claim 80 can be found in the originally filed specification at page 24, lines 14-15.

Support for new claim 81 can be found in the originally filed specification at page 24, lines 14-15.

Support for new claim 82 can be found in the originally filed specification at page 23, lines 18-19; Figure 15 at page 9, lines 8-10; Figure 49; and page 14, lines 8-19.

Support for new claim 83 can be found in the originally filed specification at page 23, lines 21-22; Figure 15; page 9, lines 8-10; Figure 49; and page 14, lines 8-19.

Support for new claim 84 can be found in the originally filed specification at page 23, lines 19-21; Figure 15; page 9, lines 8-10; Figure 49; and page 14, lines 8-19.

Support for new claim 85 can be found in the originally filed specification at page 115, lines 15-16; Figure 15; page 9, lines 8-10; Figure 49; and page 14, lines 8-19.

Support for new claim 86 can be found in the originally filed specification at page 89, lines 5-9.

Support for new claim 87 can be found in the originally filed specification at page 92, lines 27-28.

Support for new claim 88 can be found in the originally filed specification at page 92, lines 28-29.

Support for new claim 89 can be found in the originally filed specification at page 22, line 23; and page 30, line 26.

Support for new claim 90 can be found in the originally filed specification at page 22, line 24; and page 30, line 26.

Support for new claim 91 can be found in the originally filed specification in Figure 3 at page 6, lines 18-23.

Support for new claim 92 can be found in the originally filed specification at page 22, line 24.

Support for new claim 93 can be found in the originally filed specification at page 92, lines 29-30.

Support for new claim 94 can be found in the originally filed specification at page 23, lines 18-19.

Support for new claim 95 can be found in the originally filed specification at page 23, lines 19-21.

Support for new claim 96 can be found in the originally filed specification at page 23, lines 22-21.

Support for new claim 97 can be found in the originally filed specification in Figure 1B; and page 6, lines 13-14.

At the outset, applicants again convey their appreciation for the attention and feedback provided on present prosecution issues during the interviews in June 23 and June 24, 2003.

In view of the changes to the claims and the following remarks, applicants respectfully request reconsideration and withdrawal of the objections and rejections of record.

ELECTION/RESTRICTION

Applicants thank the Examiner for reconsideration and withdrawal of the restriction requirement and species election.

OBJECTIONS TO THE SPECIFICATION

In paragraph 6a-b, the Examiner requested changes to the continuing data, title and specification of the subject application to correct informalities. In response, Applicants have amended the title and specification in the accompanying Substitute Specification under 37 C.F.R. §1.125(b). (Attached as Exhibit 1, a clean version of the substitute specification and, Exhibit 2, a marked up version of the substitute specification).

Applicants provide herein, a signed declaration pursuant to 37 C.F.R. §1.125(b) stating the substitute specification contains no new matter (Exhibit 3).

In paragraph 6c-e, the Examiner requested amendments to the specification to include: separate descriptions of the Brief Description of the Figures; update the ATCC address; and to amend the specification to include sequence listing indicators. In response, Applicants file a Substitute Specification.

In view of the amendments set forth in the Substitute Specification, Applicants respectfully request reconsideration and withdrawal of the objections to the specification.

Claim Rejections: 35 U.S.C. §112 2nd Paragraph

Claims 53-56, 58-76 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. In particular, the Examiner stated that the claims were indefinite for "reciting 'a method of inducing an immune response in a subject,' because the exact meaning of the phrase is not clear. What is the immune response directed against?" (January 17, 2003 Office Action, Paper No. 14, page 4, paragraph 8). While

Applicants disagree, in order to further the prosecution of the subject application, Applicants have amended the claims.

Applicants respectfully submit that the amendments to the claims overcome the Examiner's rejection of the claims under 35 U.S.C. §112. Reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. §112 is requested.

Claim Rejections: 35 U.S.C. §112 1st Paragraph

The claims were also rejected under 35 U.S.C. §112, first paragraph. (January 17, 2003 Office Action, Paper No. 14, page 4, paragraph 10). There the Examiner alleged that the claims are to "methods of inducing an immune response in a subject having a cancer expressing a PSCA protein or cells that express PSCA" and that "[w]ith the exception of SEQ ID NO:2 and 4 the specification and the art of record does not disclose any variants [of PSCA] as broadly claimed. ..."

While Applicants disagree, in the interest of furthering prosecution, Applicants have amended the claims to specify a PSCA, or fragment thereof, as set forth in SEQ ID NO: 2. With these amendments, one of ordinary skill in the relevant art would clearly recognize Applicants' possession of the claimed invention at the time of filing. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection of the claims.

Claims 53-56 and 58-73 were further rejected under 35 U.S.C. §112, first paragraph (January 17, 2003 Office Action, Paper No. 14, page 4, paragraph 11). The Examiner stated that "while being enabling for a method of inducing an immune response against the protein of SEQ ID NO:2 in a human cancer patient that expresses the protein of SEQ ID NO:2 or cells that express SEQ ID NO:2, wherein the method comprises administration of residues 1 to 123 of SEQ ID NO:2 or a method for inducing an immune response against SEQ ID NO:4 in a mouse that expresses the protein of SEQ ID NO:4 wherein the method comprises administration of residues 1 to 123 of SEQ ID NO:4, [it] does not reasonably provide enablement for a method of inducing an immune response against just any antigen in just any subject by administration of just any portion of SEQ ID NO:2 or 4 or a method of inducing an anti-tumor response in any subject with just any

fragment of SEQ ID NO:2 or 4." (January 17, 2003 Office Action, Paper No. 14, pages 5-6).

Applicants disagree. For example, the prior claims were not limited to anti-tumor responses. However, in the interest of furthering prosecution of the subject application, and advancing towards allowance, applicants have amended the claims.

Specifically, claim 53, as herein amended, recites the use of the PSCA of SEQ ID NO:2, or a portion thereof, to induce an immune response in a human subject and claim 79, for example, specifies a humoral immune response. Respective dependent claims, further define specific portions of the sequence that may be used for the claimed methods. With the amendments to claim 53 and specification, as filed, one of skill in the art would readily be able to use the protein of SEQ ID NO:2, or portions thereof, to induce an immune response in humans. Of particular note, cancer is not an element in any claims in this matter.

For the record Applicants note that the Examiner cites Spitler demonstrative of the failure of tumor vaccines. The Examiner quotes Spitler, stating "cancer vaccines don't work," (i.e. are not clinically effective) (January 17, 2003 Office Action, Paper No. 14, page 8). However, for the purposes of the §103 rejection (discussed below), the Examiner takes an inconsistent position and uses Spitler to argue that "methods of delivering antitumor vaccines with tumor associated antigens...as well ask known methods of delivering said tumor antigens to stimulate antitumor responses" were known in the art. (January 17, 2003 Office Action, Paper No. 14, page 11). The Examiner cannot have it both ways.

DIFFERENT REJ

Applicants would like to point out that Spitler and Ezzell are both now eight years old, and do not reflect the state of the art as it relates to tumor vaccines now, or at the time of filing this application. Nevertheless, neither Spitler nor Ezzell is relevant in this §112 rejection because the claims are directed to methods for inducing an immune response.

For example, claim 53, as herein, recites "A method for inducing a cellular immune response in a human subject" and claim 79 specifies a humoral immune response. Whether or not vaccine therapy works clinically, is not a relevant part of the

determination of whether the methods of the invention work as claimed and hence are enabled. In fact the invention is operative. The specification provides data confirming methods for inducing immune responses (specification at page 85, lines 1-15 and lines 29-31; page 86, lines 1-18; Figures 12a; Figures 12b; Figure 35; Figure 21; Figure 28; page 89, lines 1-24; page 90, lines 12-30; page 91, lines 1-31; page 92, lines 1-23; page 93, lines 4-30; page 95, lines 18-31; page 96, lines 1-7).

Accordingly, applicants respectfully request reconsideration and withdrawal of the rejection of claim 53-56 and 58-73 under 35 U.S.C. §112, first paragraph. Applicants submit that with these claims are in condition for allowance and issuance of a notice of allowance is solicited.**Claim Rejections: 35 U.S.C. §103(a)**

The Examiner rejected claims 53-55 and 73-76 under 35 U.S.C. § 103(a), as allegedly obvious over Au-Young U.S. Patent No. 5,856,136 (the '136 patent,) in view of Spitler U.S. Patent No. 5,738,867 (the '867 patent). The Examiner contends that it would have been obvious to modify the methods of inducing an immune response in a bladder cancer patient in view of Au-Young and Spitler, because Au Young teaches the SCAH-2 protein is expressed in bladder tumor.

Applicants respectfully disagree and traverse the rejection on several bases.

As recited, for example in claims 53, 77 and 78, the invention is directed to:

methods for inducing a cellular immune response in a human subject having a cancer expressing a Prostate Stem Cell Antigen (PSCA) protein, comprising administering to the subject a PSCA protein fragment comprising a portion of a PSCA protein of Fig. 1B (SEQ ID NO:2); and inducing a cellular immune response directed to cells that express PSCA protein of Fig. 1B (SEQ ID NO:2). and

methods for inducing an immune response in a subject, comprising administering to the subject a PSCA protein fragment comprising a portion of a PSCA protein of Fig. 1B (SEQ ID NO:2).

Applicable Legal Standards

As set forth in detail herein and in the attached Declaration, the information in Au Young was not sufficient to expressly or inherently disclose the relevant nucleic acids and proteins in a scientifically credible way to the public. Not being disclosed in a scientifically credible way, these "compositions" were not available to the public as a basis for anticipation and add nothing of meaning to any obviousness assessment. What was disclosed and thus in possession of the public[1] is addressed by the Declaration of Dr. Steven B. Kanner pursuant to 37 C.F.R. §1.132 (herein attached as Exhibit 4). As discussed herein, as prior art, it is under the standards of 35 U.S.C. 103 (and indirectly under the standards of 35 U.S.C. 112 by means of the 103 rejection) that the Au Young reference properly is to be viewed.

To be obvious, all the elements of the invention must be found in a combination of references, where the references themselves indicate the desirability of that combination.[2] The U.S. Supreme Court addressed obviousness determinations in Graham v. Deere.[3] Thus, the issue of obviousness is to be answered by use of the inquiries set forth in Graham v. Deere. These inquiries address:

(1) the scope and content of the prior art,

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[1] See, In re Brown 51 CCPA 1254, 1259 (C.C.P.A. 1964) stating, e.g., "[w]e think , rather that the true test of any prior art relied on to show or suggest that a chemical compound is old , is whether the prior art is such as to place the disclosed 'compound' in the possession of the public"

[2] See, e.g., M.P.E.P. 2141, and Hodash v. Block Drug 786 F. 2d 1136, 1143 (Fed. Cir. 1986); In re Dance, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998); In re Gordon, 733 F.2d 900, 902, 221 U.S.P.Q. 1125, 1127 (Fed. Cir. 1984). "Combining prior art references without the evidence of such a suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability -- the essence of hindsight." In re Dembiczak, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed Cir. 1999).

[3] 383 U.S. 1148 U.S.P.Q. 459 (1966)

(2) the differences between the art and the claims at issue,

(3) the level of ordinary skill in the art, and

(4) whatever objective evidence may be present.[4]

A reasoned resolution of the basis for upholding or invalidating patents is mandated by Graham v. Deere.

There are no all-purpose criteria for applying the law of obviousness to every factual situation.[5] No one precedent or rationale can be controlling in all possible areas of human creativity. However, a uniform application of the law of obviousness is essential to the commercial incentive that is the core of the patent system. Thus it is imperative that the law be applied consistently for each invention. All relevant facts must be considered, while recognizing that it is inappropriate to squeeze new factual situations into preestablished pigeonholes.[6]

Section 103 demands that obviousness be tested as of the time of the invention was made. [7] The analytical focus is upon the state of knowledge at the time the invention was made. [8] Indeed, it has been said that facts determinable only after the invention was made may serve to evidence nonobviousness, but not obviousness.

In the present case what must be determined is whether the claimed invention would have been obvious to those who knew only what was disclosed in the prior art.[9] The prior art must be viewed without reading into it the applicant's teachings.[10] The Federal Circuit regards hindsight as an insidious and powerful phenomenon and insists upon use of the statutory phrase "would have been obvious," which tends to remind the

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[4] Specialty Composites v. Cabot Corp., 845 F.2d 981, 6 USPQ2d 1601 (Fed.Cir.1988); Allen Archery, Inc. v. Browning Mfg. Co., 819 F.2d 1087, 2 USPQ2d 1490 (Fed.Cir.1987); Connell v. Sears, Roebuck & Co., 722 F.2d 1542, 220 U.S.P.Q. 193 (Fed.Cir. 1983).

[5] See, e.g., Harmon, Patents and the Federal Circuit, 6th ed. § 4.2 *et seq* beginning at 153 (2003, BNA, Washington, D.C.)

[6] In re Eli Lilly & Co., 902 F.2d 943, 14 USPQ3d 1741 (Fed.Cir.1990)

[7] 35 U.S.C. §103

[8] In re Raynes, 7 F.3d 1037, 28 USPQ2d 1630 (Fed.Cir.1993)

[9] Panduit Corp. v. Dennison Mfg. Co., 774 F2d 1082, 227 U.S.P.Q. 337 (Fed.Cir.1985); Rosemount, Inc v. Beckman Instruments, Inc., 727 F.2d 1540, 221 USPQ1 (Fed.Cir.1984)

[10] Vandenberg v. Dairy Equip. Co., 740 F.2d 1560, 224 U.S.P.Q. 195 (Fed.Cir.1984); Kansas Jack, Inc. v. Kuhn, 719 F.2d 1144, 219 U.S.P.Q. 857 (Fed.Cir.1983)

decisionmaker of the need to cast the mind back to the time the invention was made.[11] The obviousness standard requires the decisionmaker to return to the time the invention was made.[12] The Federal Circuit forecloses the use of hindsight, noting that the statute commands that the obviousness inquiry is restricted to the knowledge available in analogous areas of endeavor at the time the invention was made in strict ignorance of subsequent developments that would permit the invention to be constructed through hindsight.[13] Similarly, under anticipation one cannot use hindsight to attribute existence of elements of an invention to a single prior art disclosure. Hindsight is a tempting but forbidden zone.[14]

Further, as set forth in M.P.E.P. 2145(j)(1), any rejection for obviousness must take into account only knowledge which was within the level of ordinary skill in the art at the time that the claimed invention was made, and not knowledge gleaned from applicants' disclosure of his invention.[15] It is further explained in M.P.E.P. 2141.01(c), the "[r]equirement for 'at the time the invention was made' is to avoid impermissible hindsight." Hence, any combination of references must be suggested in the references themselves, not in the application that is being examined.

Obviousness Not Established by Merely Obvious to Try

It is well settled that "obvious to try" is not a standard that establishes that an invention is obvious. The fact that known principles are employed does not make an invention obvious; most patentable inventions employ known principles."[16] Nor does obviousness necessarily follow from a conclusion that the invention is but the product of

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[11] Panduit Corp. v. Dennison Mfg. Co., 774 F.2d. 1082, 227 U.S.P.Q. 337 (Fed.Cir. 1985)

[12] Uniroyal, Inc v. Rudkin-Wiley Corp., 837 F.2d 1044, 5 USPQ2d 1434 (Fed Cir. 1998)

[13] W.L. Gore & Assocs. v. Garlock, Inc., *supra* (Fed Cir. 1983).

[14] Interconnect Planning Corp. v. Feil, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed.Cir. 1985); Panduit Corp. v. Dennison Mfg. Co., 774 F2d 1082, 227 U.S.P.Q. 337 (Fed. Cir. 1985)

[15] The earliest filing of the present PSCA invention occurred 10 March 1997. Therefore, all determinations of whether claim elements were a) in the art, b) enabled and c) in properly combinable is determined as of this date.

[16] Lindemann Maschinenfabrik v. American Hoist & Derrick Co., 730 F.2d 1452, 221 U.S.P.Q. 481, 489 (Fed. Cir. 1984).

the "natural evolution of the prior art."[17] "A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out."[18]

The Federal Circuit has repeatedly emphasized that "obvious to try" is not a standard that leads to a decision of obviousness under §103. Nevertheless, the meaning of the maxim can sometimes be elusive. The fundamental question is: "When is an invention that was obvious to try nevertheless nonobvious?"[19] The Federal Circuit postulates three obvious to try situations that might lead to a conclusion of obviousness:

(1) to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful;

(2) to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it; and

(3) where the reference contained detailed enabling methodology for practicing the claimed invention, and evidence suggesting that it would be successful.

According to the Federal Circuit,[20] the first two situations do not result in obviousness, whereas only the third does.

Standards for Enablement and Description of Prior Art References

As noted, in order for a prior art reference to serve as a bar to patentability it must

[17] State Indus., Inc. v. Rheem Mfg. Co., 769 F.2d 762, 227 U.S.P.Q. 375 (Fed. Cir. 1985).
[18] In re Deuel, 51, F.3d 1552, 34 USPQ2d 1210 (Fed. Cir. 1995).
[19] In re O'Farrell, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1986). *See also*, M.P.E.P. §2145(X)(B).
[20] In re O'Farrell, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988).

be enabling. The enablement requirement is set forth in 35 U.S.C. § 112, ¶ 1, which also contains the written description requirement. The enablement and written description requirements are distinct, yet related. The purpose of the written description requirement is to state what is needed to fulfill the enablement requirement,[21] and assures that an invention be described in a manner that makes it clear an inventor actually invented what is claimed. 35 U.S.C. § 112, ¶ 1 provides:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same....

Thus, an applicant's written description must communicate that which is necessary to enable a skilled artisan to make and use the claimed invention. Similarly, a prior art description must place the skilled artisan in possession of the invention. A written description that does not meet this requirement is legally insufficient.[22] These fundamental concepts are very important when contemplating any effect of Au Young as prior art.

The enablement requirement "demands that the patent specification enable 'those skilled in the art to make and use the full scope of the claimed invention without undue experimentation.'" [23] Thus, another issue that will be evaluated here is whether Au

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[21] Harmon, Patents and the Federal Circuit, 6th ed. § 5.2(a) at 218 (2003, BNA, Washington, D.C.) "The description requirement is different from enablement and requires that the invention be described in such a way that it is clear that the applicant invented what is claimed. A patent discloses only that which it describes, whether specifically or in general terms, so as to convey intelligence to one capable of understanding. The purpose of the description requirement is to state what is needed to fulfill the enablement criteria. The written description must therefore communicate that which is necessary to enable the skilled artisan to make and use the claimed invention. A description that does not do this is legally insufficient." (citations omitted)

[22] Kennecott Corp. v. Kyocera Int'l., Inc. 835 F.2d 1419, 5 USPQ2d 1194 (Fed Cir 1987); cert. den. 486 U.S. 1008 (1988).

[23] National Recovery Technologies, Inc. v. Magnetic Separation Sys., Inc., 166 F.3d 1190, 1195 (Fed. Cir. 1999) (quoting Genentech, Inc. v. Novo Nordisk A/S, 108 F.3d 1361, 1365 (Fed. Cir.), cert. denied, 522

Young would require undue experimentation in order to practice any elements being cited against the present claims.[24] When undue experimentation is required to practice the invention a disclosure is not enabled, and it is insufficient as prior art.

Thus, before Au Young can suffice as claim-defeatingit must be determined whether Au Young requires "undue experimentation" before the disclosed and claimed "invention" could be practiced by those skilled in the art. Factors to be considered in determining whether a disclosure requires undue experimentation include:[25]

(1) the quantity of experimentation necessary,

(2) the amount of direction or guidance presented,

(3) the presence or absence of working examples,

(4) the nature of the invention,

(5) the state of the prior art,

(6) the relative skill of those in the art,

(7) the predictability or unpredictability of the art, and

(8) the breadth of the claims.

"[A]ll of the [Wands] factors need not be reviewed when determining whether a disclosure is enabling."[26] These factors "are illustrative, not mandatory. What is relevant depends on the facts".[27]

In the context of molecular biology it has been recognized that although the level of skill in the art is quite high, that experiments in genetic engineering produce, at best, unpredictable results.[28] In cases involving unpredictable art, such as biotechnology, more

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U.S. 963, 118 S.Ct. 397, 139 L.Ed.2d 310 (1997) (quoting In re Wright, 999 F.2d 1557, 1561 (Fed.Cir.1993)) (internal quote omitted).

[24] See, e.g., M.P.E.P. 2121.01 (II) concerning use of nonenabled prior art under 35 U.S.C. 103 to determine obviousness.

[25] In re Wands 858 F.2d 731, 737 (Fed Cir 1988).

[26] Enzo Biochem, Inc. v. Calgene, Inc. ("Calgene"), 188 F.3d 1362, 1371 (Fed.Cir.1999)

[27] Amgen, Inc. v. Chugai Pharm. Co., Ltd., 927 F.2d 1200, 1213 (Fed. Cir.), *cert. denied,* 502 U.S. 856, 112 S.Ct. 169, 116 L.Ed.2d 132 (1991)

[28] The Board *Ex parte Forman* recognized that "the level of skill in the art of molecular biology is quite high'" but also that "experiments in genetic engineering produce, at best, unpredictable results." The specification and a publication cited by the applicant there failed to provide guidance as to either how many

disclosure is required in order to enable.[29] There must be some disclosure or guidance leading to predictability. If there are precisely reproducible working examples, this is a factor for consideration on enablement, and logically it can be an important one.

The issue of whether elements of an invention are actually disclosed in a reference is addressed by the predecessor to the Federal Circuit:[30]

The proper test of a description in a publication as a bar to a patent as the clause is used in 102(b) requires a determination of whether one skilled in the art to which the invention pertains could take the description of the invention in the printed publication and combine it with his own knowledge of the particular art and from this combination *be put in possession of the invention on which a patent is sought.* Unless this condition prevails, the description in the printed publication is inadequate as a statutory bar to patentability under section 102(b).

Written Description

For guidance on description issues vis-à-vis prior art, we review the principles of written description as they have been developed under 35 U.S.C. 112. Under the principle of written description, a patent discloses only that which it describes, whether specifically or in general terms, so as to convey intelligence to one capable of understanding.[31] A specification must describe every element of the claimed invention in sufficient detail so that one of ordinary skill in the art would recognize that the inventor

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mutant strains are formed in each experiment or "how much time and effort and what level of skill must be exercised to isolate the single strains which are then cloned to yield the useful vaccines." The applicant admitted that it was estimated that it would take "about one year to construct" most strains according to the invention. The Board stressed that "time is not the sole criterion." However, "in the present case there is also a lack of guidance leading to predictability." Finally, the Board noted that "[t]here do not appear to be apparently precisely reproducible working examples." In denying the applicant's request for reconsideration, the Board emphasized that "the absence of a working example was not in itself stated to cause the specification to be insufficiently enabling." Such absence was merely one factor. Ex parte Forman, 230 U.S.P.Q. 546, 547 (Bd. Pat App. & Int. 1986).

[29] See, e.g., In re Wright 999 F2d. 1557 (Fed Cir 1993)

[30] In re Samour, 197 U.S.P.Q. 1 (C.C.P.A. 1978); See also In re Donohue, 207 U.S.P.Q. 196 (C.C.P.A. 1980)

[31] In re Benno 768 F2d 1340, 226 U.S.P.Q. 683, 686 (Fed Cir 1985).

possessed the claimed invention at the time of filing.[32] The Federal Circuit indicates that a description must allow persons of ordinary skill in the art to clearly conclude that "the inventor invented the claimed invention."[33]

Under written description, the issue is not whether a disclosure enables one of ordinary skill in the art to make the later claimed invention, i.e., "it is not a question of whether one skilled in the art *might* be able to construct the patentee's device from the teachings of the disclosure."[34] Instead, the "description must be sufficiently clear that persons of skill in the art will recognize that the applicant made the invention having those limitations."[35] Thus, one issue that will be evaluated here is whether Au Young contains a legally sufficient description of any elements being cited against the present claims.[36] A disclosure that fails to meet the description requirement is not enabled and is insufficient as prior art.

The Court of Appeals for the Federal Circuit has on more than one occasion discussed the written description requirement as it applies to biotechnology patents, particularly those in which a substance is defined by a description of its function or the desired result of its use.

For example, in Fiers v. Revel,[37] the court held (in the context of an interference proceeding) that a patent application failed to satisfy the written-description requirement where the claimed invention called for a "DNA which codes for a human fibroblast interferon-beta polypeptide" ("β-IF"), and the application disclosed a method for isolating a fragment of the DNA coding for β-IF as well as a method for isolating messenger RNA coding for β-IF, but did not disclose a complete DNA sequence coding for β-IF. In reaching that holding, the court stated that "[a]n adequate written description of a DNA

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[32] Vas-Cath, 935 F.2d at 1563; In re Alton 76 F3d 1168, 1172; 37 USPQ2d 1578, 1581 (Fed Cir 1996).

[33] Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572 (Fed.Cir.1997) See also, In re Gosteli, 872 F.2d 1008, 1012 (Fed.Cir.1989).

[34] Martin v. Mayer 823 F.2d 500, 505, 3 USPQ2d 1333, 1337 (Fed Cir 1987)

[35] Id.

[36] See, e.g., M.P.E.P. 2121.01 (II) concerning use of nonenabled prior art under 35 U.S.C. 103 to determine obviousness.

[37] Fiers v. Revel, 984 F.2d 1164 (Fed.Cir.1993).

requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it; what is required is a description of the DNA itself."[38] The court stated that the disclosure contained in the application "just represents a wish, or arguably a plan, for obtaining the DNA," and that "it does not indicate that [the applicant] was in possession of the DNA."[39] The court added that a description of DNA requires "a precise definition, such as by structure, formula, chemical name, or physical properties ...." The court said that "[c]laiming all DNA's that achieve a result without defining what means will do so is not in compliance with the description requirement; it is an attempt to preempt the future before it has arrived."[40].

The Federal Circuit revisited this area in Enzo Biochem, Inc. v. Gen-Probe Inc.[41] ("Enzo"). In Enzo, the district court had concluded that the claimed composition was defined only by its biological activity or function, which it held was insufficient to satisfy § 112, paragraph 1. The Federal Circuit determined that its initial ruling that a deposit may not satisfy the written description requirement was incorrect.[42] In so holding, however, the court did not overturn its prior case law that "a mere wish or plan" for obtaining an invention is not enough to comply with § 112, paragraph 1.[43] While the court did state that "[i]t is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement",[44] the court did *not* hold that all such functional descriptions *are* sufficient. Rather, the court adopted the standard set forth in the Patent and Trademark Office ("PTO") Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, 1 "Written Description" Requirement ("Guidelines"), 66 Fed. Reg. 1099 (Jan. 5, 2001), which state that the written description requirement can be met by "showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics," including, *inter alia,*

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[38] Fiers v. Revel, 984 F.2d 1164, 1170 (Fed.Cir.1993).
[39] Id. at 1171.
[40] Id.
[41] Enzo Biochem, Inc. v. Gen-Probe Inc. 323 F.3d 956 (Fed.Cir.2002)
[42] Enzo, 323 F.3d 956 (Fed.Cir.2002).
[43] Id. at 964 (quoting Eli Lilly, 119 F.3d at 1566)
[44] Id.

"functional characteristics when coupled with a known or disclosed correlation between function and structure ...."[45] (emphasis original).

Applicants' Invention Is Nonobvious Over the cited art

The Au-Young Reference

Au Young teaches multiple SCAH-2 assemblies based on arranging overlapping transcript fragments found in both normal uterine and bladder tumor cells. However, Au-Young is a defective and deficient disclosure of the SCAH-2 assemblies which it discloses for the reasons which follow and are set forth in the attached Declaration by Dr. Kanner.

Patent law often involves subject matter and legal principles that can be both complex and arcane. But there are some basic principles that should be evident even to the lay person. Such a lay person is particularly relevant when they are one of ordinary skill in the relevant art, and the question they are presented with is whether elements of an invention are disclosed to them. Once again, in order for a prior art reference to have patent-defeating meaning, it must place subject matter sought to be patented into possession of the public.

Similarly, an "inventor" or patentee is entitled to a patent to protect his work but only if he produces or has possession of the subject matter being claimed. The subject matter he claims must be sufficiently concrete so that it can be described for the world to appreciate the specific nature of the work that sets it apart from what was before. The inventor must be able to describe the subject to be patented with such clarity that the reader is assured that the inventor actually has possession and knowledge of the unique composition or method that makes it worthy of patent protection. The Au Young patent does not do that.

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[45] Enzo, 956 F.3d at 964 (quoting Guidelines, 66 Fed. Reg. at 1106 (emphasis original to case)).

The Au Young patent teaches a wish or plan for obtaining a desired result. But Au Young failed to do what was necessary to accomplish the desired end. Thus, such a teaching does not rise to an "invention." As the Court of Appeals for the Federal Circuit stated in a case involving similar issues, an inadequate patent description that merely identifies a plan to accomplish an intended result "is an attempt to preempt the future before it has arrived."[46]

Au Young is just such a disclosure, it fails to meet the requirements of the federal statutes concerning sufficiency as a prior art reference. Moreover, as regards PSCA subject matter, Au Young is not enabled prior art since undue experimentation was needed before one of ordinary skill in March 1997 could practice the PSCA subject matter. Thus, any assertion of unpatentability based on such a reference must fail.

Au Young has been cited as art disclosing compositions asserted to have functional significance, thus a question of written description sufficient to support enablement of that prior art arises. Applying principles of written description and enablement to the cited art, Applicants submit that the Au Young reference fails to meet both the written-description and enablement requirements of § 112. Au Young does no more than describe a putative gene, ambiguously attempts to list a protein(s), and asserts a function for the protein(s) and prophetically asserts that antibodies are made to it. However, Au Young contains no information by which a person of ordinary skill in the art would understand that Au Young possessed the claimed invention, a gene that encodes a SCAH-2 protein, or that protein, or any antibodies that bind thereto. At best, the Au Young disclosure indicates that one could run a number of tests in the hope that a whole panoply of prophetically stated assumptions occur.

Nowhere in the Au Young disclosure is there even a suggestion that the SCAH-2 protein existed in a cell or sample let alone that it would be suitable for use in practicing the claimed invention. Instead, the disclosure about SCAH-2 insofar as its sufficiency for purposes of the written-description requirement is concerned, creates a situation analogous to the one addressed by Lilly, where the court stated that "[t]he description

[46] Fiers v. Revel, 984 F.2d 1164, 1171 (Fed.Cir.1993).

requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention."[47]

Furthermore, to the extent that the Enzo 323 F.3d 956 (Fed.Cir.2002) court held that a functional description can meet the written-description requirement, it did so in accordance with PTO guidelines stating that the requirement can be met by disclosing "sufficiently detailed, relevant identifying characteristics," including "functional characteristics when *coupled* with a known or disclosed correlation between function and structure ...." No such correlation has been disclosed by Au Young.

Consistent with that standard, the central holding of Enzo was that with respect to "biological materials"[48], "reference in the specification to a deposit in a public depository, which makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the written description requirement of § 112, ¶ 1."[49] In other words, it is not necessary to give a precise chemical formula or description of a chemical structure when persons of ordinary skill in the art can ascertain what substance is being described by resort to a public depository where a specimen of that substance is kept. Au Young made no such deposit.

It is necessary that the patent set forth enough detail to allow a person of ordinary skill in the art to understand what is claimed and to recognize that the inventor invented what is claimed. This Au Young fails to do. Absent this description the subject matter is not enabled.

The citation of Au Young as prior art, as well as the Au Young claims, *requires that* there be a protein in existence, i.e., present in some cell or sample.[50] Without

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[47] 119 F.3d at 1568. Moreover, the court cited in support of that statement, In re Wilder, 736 F.2d 1516 (Fed.Cir.1984), cert. denied, 469 U.S. 1209, 105 S.Ct. 1173, 84 L.Ed.2d 323 (1985), which did not involve any chemical-related claims but claims directed to a mechanism for indicating the location of information recorded on a dictating machine. The Wilder court affirmed the PTO's rejection of those claims because the specification did "little more than outlin[e] goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate." Id. at 1521.

[48] Id. at 965

[49] Id.

[50] See, e.g., footnote *** and related text re the need to provide this information to the public.

existence of the protein, it is impossible to 1. make any conclusion regarding whether subject matter is disclosed in the art or 2. to practice any relevant claim; the protein must exist in order for the claim to have utility. It means little to "invent" or disclose a gene if one does not have possession of the resulting protein. Possession of the protein is essential to utility of the gene. Without that protein the claimed invention is more theoretical than real; it is akin to "inventing" a cure for cancer by utilizing a substance that is not known to exist in a cell or sample but if found allegedly will attack and destroy cancer cells while leaving healthy cells alone. Without possession of such a substance, such a "cure" is illusory, and there is no meaningful or scientifically credible possession of the method. There are quite simply no elements described in the Au Young disclosure that disclose to or allow one of skill in the art to practice the claims of present invention; this conclusion is confirmed by the attached Declaration of Dr. Steven B. Kanner.

A hindsight view of Au Young in view of Applicants' discovery of PSCA is not how the obviousness of PSCA should be viewed. Instead Au Young should be considered as it would have in the context of March 1997. Au Young contemplated that there *may* be a SCAH-2 gene in nature, there *may* be a SCAH-2 protein, and that the protein *may* have some sort of function or disease correlation. Although Au Young found part of SCAH-2 in bladder tumor, Au Young does not show a correlation of SCAH-2 to any type of cancer. Indeed Au Young does not show any expression data that would indicate that SCAH-2 assembly levels exist or are irregular in any of the tissue Au Young assayed. These are important steps in the inventive process.

What Au Young *did not do*, is succeed in taking the fundamental and critical steps of actually isolating such a gene, finding such a protein, identifying any meaning to such protein, or raising antibodies to any such protein. Absent these steps, Au Young's explorations, valuable though they might have been in *hindsight*, did not blossom into a complete, described and enabled subject matter. Scientific developments, and theories based on them frequently lay the groundwork for later inventions, but that does not make the developer an inventor as well.

At most, the Au Young disclosure specifies the need for further trial and error.

Knowing some "starting point" is not enough; that is little more than a research plan. Here the actual starting materials are not even provided in the art.[51] The patent does not specifically describe the importance of confirming the existence of the gene and protein in any cell or sample.

As confirmed in the attached Declaration, what Au Young does *not* provide to one of skill in the art is an understanding that the SCAH-2 assembly and protein are truly in any cell or sample. One would not know or expect that any information except through trial and error, which hardly suggests possession of a complete, enabled disclosure. The Au Young patent disclosure concerning SCAH-2 was hypothetical.

The Au Young disclosure, and its multiple nucleic acid assemblies, was confusing at best. Section 112, ¶ 1, however, requires the patentee to "show that an invention is *complete* by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was *in possession* of the claimed invention ...."[52] "[T]he disclosure must show [that the inventor] had invented *each feature* that is included as a claim limitation" (emphasis added).[53] Without possession, or at least knowledge that any of the multiple SCAH-2 assemblies existed, i.e., were present in any cell or sample, Au Young could not have possessed in a scientifically credible way a SCAH-2 gene, a SCAH-2 protein, antibodies to SCAH-2, relevant expression data of SCAH-2 protein, and/or relevant uses of any of these.

"[T]he applicant must ... convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention".[54] The "[written description] requirement is not satisfied if one of ordinary skill in the art must first make the patented invention before he can ascertain the claimed features of that

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[51] This differs from a case such as Univ. of Rochester v. G.D. Searle & Co. 249 F Supp.2d 216 (W.D. N.Y. 2003) where there was a scientific foundation, the existence of two prostaglandin activities, PGHS-1 and PGHS-2. In Rochester the claimed subject matter, a method of using selectively inhibiting PGHS-2, was found to not be legally described because the selective binding composition for use in the method did not exist. By analogy, Au Young does not even go as far as establishing that the two PGHS exist, it *merely speculates* that they do.

[52] Guidelines, 66 Fed. Reg. at 1106 (emphasis added)

[53] New Railhead Mfg., L.L.C. v. Vermeer Mfg. Co., 298 F.3d 1290, 1295 (Fed.Cir.2002)

[54] *See* Vas-Cath, 935 F.2d 1555, 1563-64

invention".[55] As confirmed by the attached Declaration, Au Young did not do the former, and the later was required before one of skill could attribute any meaning to the disclosure.

In effect, then, Au Young "discloses", and in some aspects claims, subject matter that cannot be practiced until one discovers actual existence and relevant expression of the subject matter, however that information was neither disclosed by Au Young nor otherwise known in the art. Putting Au Young's disclosure, or claims, into practice awaited someone actually discovering necessary components of an invention.

The Federal Circuit in Fiers made the following observation about "[t]he difficulty that would arise if we were to hold that a conception occurs when one has only the idea of a compound, defining it by its hoped-for function"[56]:

> would-be inventors would file patent applications before they had made their inventions and before they could describe them. That is not consistent with the statute or the policy behind the statute, which is to promote disclosure of inventions, not of research plans. While one does not need to have carried out one's invention before filing a patent application, one does need to be able to describe that invention with particularity.

That concern is well illustrated by Au Young, and its use as a prior art reference. Subject matter which Au Young could not even describe in a scientifically credible way is not enabled.

In addition to lacking enablement for failure to describe subject matter under the written-description requirement, Au Young does not comply with the enablement requirement of § 112 on other bases. To practice the subject matter invention in Au Young, some of which is claimed, a person of ordinary skill in the art would have to engage in undue experimentation, with neither assurance nor even a reasonable

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[55] *New Railhead Mfg.*, 298 F.3d at 1295

[56] Fiers, 984 F.2d at 1169

expectation of success. PSCA assembly, proteins thereof, and uses thereof are not enabled by Au Young.

The fact that some data or experimentation is called for does not *per se* mean that the patent fails to meet the enablement requirement.[57]

It must be remembered, however, that "[p]atent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable. Tossing out the mere germ of an idea does not constitute enabling disclosure."[58] "[A] patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion".[59] Thus, while the need for some experimentation is by no means necessarily fatal, "reasonable detail must be provided in order to enable members of the public to understand and carry out the invention."[60] As set forth in the attached Declaration, such reasonable detail is lacking in Au Young.

Au Young prophetically describes incomplete gene and protein sequences and prophetically proposes protein expression. However, it does not identify that the gene or protein in any cell or sample. Omitting this vital information, Au Young then describes some of the steps to be taken *when* such crucial elements and expression data are identified. The necessary link was not available: actually finding the SCAH-2 gene and protein in any cell or sample, and identifying relevant protein function or expression.

Au Young provides "intimations of general ideas that may or may not be workable"[61] is provided, yet nowhere does Au Young set forth that the elements exist in a cell or sample for practicing the present invention. Just as concluded by the Federal Circuit,[62] tossing out the mere germ of an idea does not constitute enabling disclosure.

It is not sufficient to contend that Au Young could properly omit detail in this

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[57] *See* W.L. Gore & Associates, Inc. v. Garlock, Inc., 721 F.2d 1540, 1557 (Fed.Cir.1983) (district court erred in invalidating patents for indefiniteness because of its view that some "trial and error" would be needed), *cert. denied,* 469 U.S. 851, 105 S.Ct. 172, 83 L.Ed.2d 107 (1984).

[58] Genentech, 108 F.3d at 1366

[59] Brenner v. Manson, 383 U.S. 519, 536, 86 S.Ct. 1033, 16 L.Ed.2d 69 (1966) (stated in context of the utility requirement)

[60] Id.

[61] Genentech v. Novo Nordisk, 108 F3d 1361, 1366 (Fed. Cir. 1997)

[62] Id.

regard, on the basis that the procedures for finding the sought-after materials and data were well known in the art. While it is true that a patent need not disclose that which is already well known in the art in order to be enabling,[63] the Federal Circuit has cautioned:[64]

> that general, oft-repeated statement is merely a rule of supplementation, not a substitute for a basic enabling disclosure. It means that the omission of minor details does not cause a specification to fail to meet the enablement requirement. However, when there is no disclosure of any specific starting material or of any of the conditions under which a process can be carried out, undue experimentation is required; there is a failure to meet the enablement requirement that cannot be rectified by asserting that all the disclosure related to the process is within the skill of the art. [emphasis added]

Here, far more than "minor details" are missing and undue experimentation is required to complete enablement of the Au Young disclosure. As described in detail below, missing data include crucial elements of all aspects of a useful, novel, nonobvious, enabled, gene, protein and antibodies to the protein. These glaring omissions cannot, for example, be rectified by an assertion that the necessary information would have been known or determinable by one of skill in the art.[65]

In short, although Au Young placed words on paper concerning SCAH-2 gene, protein and antibodies to such (and other) proteins, and identifies some broad categories of disease where SCAH-2 (and others) may be relevant, these descriptions, without more precise guidelines, amount to little more than "a starting point, a direction for further research."[66] "[T]he teachings set forth in the specifications provide no more than a 'plan'

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[63] *See, e.g.*, Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1385 (Fed.Cir.1986), *cert. denied*, 480 U.S. 947, 107 S.Ct. 1606, 94 L.Ed.2d 792 (1987)

[64] Genentech, 108 F.3d at 1366, (emphasis added)

[65] Genentech, 108 F.3d at 1366. *See also* Perreira v. Secretary of Dep't of H.H.S., 33 F.3d 1375, 1377 n. 6 (Fed.Cir.1994) ("An expert opinion is no better than the soundness of the reasons supporting it").

[66] Genentech, 108 F.3d at 1366.

or 'invitation' for those of skill in the art to experiment practicing [the claimed invention]; they do not provide sufficient guidance or specificity as to how to execute that plan".[67] It has been noted that where a disclosure "recognizes a specific need ... and suggests a theoretical answer to that need. It provides a starting point from which one of skill in the art can perform further research in order to practice the claimed invention, but this is not adequate to constitute enablement".[68]

The Office's obviousness rejections in view of Au Young may be credible only when predicated on several serial assumptions. The assumptions that must underlie the rejection are that concerning the SCAH-2 assemblies, persons of ordinary skill in the art would have found that it would have been scientifically credible that:

1) Au Young disclosed a nucleic acid sequence that actually exists. *However, no nucleic acid sequence that was actually present in any cell or sample was ever documented by Au Young.*

2) Au Young in a scientifically credible way appreciated the correct reading frame for Applicants' PSCA protein. *However, Au Young ambiguously referred to several amino acid sequences, some based on an open reading frame and others not.*

3) Au Young actually intended a particular protein as SCAH-2. *However, Au Young ambiguously referred to several amino acid sequences, and no amino acid sequence was ever documented in any cell or sample by Au Young.*

4) A protein was truly encoded by saidassembly, i.e., that it was not a pseudogene. *However, no SCAH-2 protein was ever documented (i.e., shown to exist or expressed in any cell or sample) by Au Young.*

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[67] Calgene, 188 F.3d at 1374

[68] National Recovery Technologies, 166 F.3d at 1198

5) Any such protein actually existed in a cell or sample as if so was so scientifically credible that antibodies would be raised to it. *However, no antibodies were raised by Au Young.*

Far too much is missing from Au Young to have made the above assumptions credible to one of ordinary skill in the art in March 1997. The Au Young disclosure does not describe SCAH-2 protein in a cell or sample or any uses of such protein so as to place those of skill in the art in possession of such, nor does it enable one of ordinary skill in the art to make or use such. These conclusions are supported by the attached Declaration of Dr. Steven B. Kanner.

Only the impermissible use of hindsight[69] could lead to the conclusions of the Patent Office. Au Young lists a hypothetical gene, SCAH-2, which was a nucleotide assembly sequence. This assembly was constructed from two different tissues; the tissues were at two different states of neoplasia.[70] Au Young never constructed a gene or protein, and never confirmed the existence of any gene or protein in a cell or sample. Accordingly, Au Young could not have made any SCAH-2 deposit in a public depository. The Au Young disclosure lacks any tissue distribution, or cancer-association data. Neither the assembly nor protein in Au Young was a fully completed sequence. Thus, neither the SCAH-2 assembly nor protein set forth in Au Young match the respective PSCA sequence (of the invention in view of the sequence errors, gaps and unknown sequence positions). There was ambiguous disclosure of any open reading frame (ORF). No data confirms that the assembly is a gene in any cell or sample. There is no actual data that correlates any SCAH-2 protein with any disease, and no actual data on any protein function. Au Young made no antibodies whatsoever. Collectively, there was no motivation to make antibodies that immunospecifically recognized and bound PSCA or fragments thereof. Moreover, even if it were obvious to try and make

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[69] Such as present Applicants' disclosure of PSCA gene, protein and relevant expression.

[70] One component is bladder tumor (Au Young '391, col. 4, lines 27-29, col. 5, lines 1-18) and the other is some sort of uterus tissue, presumed to be normal for lack of any other description (Au Young '391, col. 4, lines 27-29).

antibodies to SCAH-2, there would have been no reasonable expectation of success that antibodies would have the recited characteristics without the use of hindsight based on Applicants' disclosures.

Without knowing an actual sequence, it may be possible to reduce an invention to practice by isolating a protein from a natural source or depositing a DNA that encodes the protein.[71] However, neither isolation of a protein from a cell or sample, nor a deposit was made in Au Young.

Accordingly, as related to the requirements of 35 U.S.C. § 103, the real issue here is simply whether a prophetic, generic disclosure is adequate to describe and enable so as to defeat patentability of claims to methods for inducing an immune response using the claimed PSCA of SEQ ID 2 or portions thereof. The applicants submit that where the claimed subject matter is a human gene or protein or uses thereof, and where one of skill in the art cannot even determine if the prior art even found a gene or protein that exists in a cell or sample, the prior art could not be deemed to have described and enabled the present invention in any scientifically credible manner.

The six assumptions that underlie rejection of the present claims based on Au Young will now be addressed.

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[71] See, e.g., In re Fisher 427 F2d 833, 166 U.S.P.Q. 18 (C.C.P.A. 1970) As illustrated in Fisher, written description may be satisfied by disclosure of a biological product having specific and known biological function, without any description of its structure. Such an invention would not require recitation of the nucleic acid sequence of a gene invention or the amino acid sequence of a polypeptide product. *But cf.* Fiers v. Sugano 984 F.2d 1164, 1171, 25 USPQ2d 1601, 1606 (Fed Cir 1993)

1. **Assumption: That Au Young disclosed a nucleic acid assembly that actually existed, i.e., was present in any cell or sample.**

   Fact: No nucleic acid assembly was ever documented to have been present in any cell or sample by Au Young; the sequence itself is incomplete and not fully sequenced.

Viewed as prior art in March 1997 Au Young provides a hypothetical nucleotide assembly sequence.[72] As a consequence of the fact that Au Young never established the existence of the SCAH-2 assembly (or correlative protein) in any cell or sample, the disclosure lacks any tissue distribution, or disease-association data.

The SCAH-2 nucleic acid assembly sequence was derived from six overlapping nucleic acid fragments isolated from two distinct tissues, and of two states of neoplasia.[73]

As set forth in the attached Declaration, as of March 1997 a conclusion that a SCAH-2 gene exists in nature was not scientifically credible.[74]

As stated in the attached declaration, asserting existence of a gene in nature on the basis of a fragment assembly is highly suspect for a number of reasons.[75] For example:

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[72] One component is bladder tumor (Au Young '391, col. 4, lines 27-29, col. 5, lines 1-18) and the other is some sort of uterus tissue, presumed to be normal for lack of any other description (Au Young '391, col. 4, lines 27-29).

[73] The Au Young nucleotide sequence was assembled from the overlapping sequences found amongst the six Incyte Clones 588615, 590328, 1312529, 1314679, 1315052 and 1317088. Au Young '136, column 4, lines 50-59. These clones are designated in Au Young as SEQ ID NOS: 21-26. Thus, the Au Young nucleic acid assembly sequence was made using:

SEQ ID 21—normal uterus library
SEQ ID 22—normal uterus library
SEQ ID 23—bladder tumor library
SEQ ID 24—bladder tumor library
SEQ ID 25—bladder tumor library; and
SEQ ID 26 —bladder tumor library

[74] See, e.g., Sorek and Safer, "A Novel Algorithm for Computational Identification of Contaminated EST Libraries" Nucl. Acids Res 31(3): 1067-1074 (2003)

[75] See, e.g., Sorek and Safer, "A Novel Algorithm for Computational Identification of Contaminated EST Libraries" Nucl. Acids Res 31(3): 1067-1074 (2003)

- One or more of the short fragments used to construct the SCAH-2 assembly could have sequence errors, making the assembly and subsequences thereof artifacts.
- The fragments could contain or be contaminant sequence such as vector, linker, or virus.
- The fragments could be chimeric sequences. A chimera is a concatenation of two or more expressed sequences from different areas. If a chimera is used it will likely result in combination of two genes into a single incorrect gene prediction.
- ESTs can also be a contaminant of genomic DNA from the organism itself. Genomic contaminants can make introns appear to be expressed.
- Even if an individual nucleic acid fragment used in the assembly does code for a protein, that protein may not be SCAH-2. Instead, the EST could be spliced into a variant gene that codes for some other protein.
- Since the fragments used to construct the assembly came from two tissues, it is possible that the "gene" does not actually exist in either one of those tissues.
- Premature mRNA (pre-mRNA), i.e., mRNA that did not undergo splicing is another form of EST contamination; in this situation intronic sequences will falsely appear to be exons. (Additionally, as discussed below, even if the assembly occurs in nature, it may not be a gene at all but a pseudogene).

The SCAH-2 assembly was never cloned at all, let alone cloned in a confirmatory manner that shows a full length gene.[76] Furthermore, the SCAH-2 nucleotide sequence (SEQ ID NO: 4 of Au Young) is incomplete and not fully sequenced. The nucleic acid sequence of SCAH-2 was incomplete in several respects. It had an unknown (labeled "N") at nucleic acid position 475, and listed five (5) positions on a 50% chance basis.

[76] For example, from multiple identified hits obtained by probing a sequence library with probes derived from the putative nucleic acid assembly sequence, or by PCR.

We note that SCAH-2 differs from human PSCA at both the nucleic acid sequence and amino acid sequence levels. As stated in the attached Declaration, the PSCA sequence includes 990 nucleotides, whereas SCAH-2 includes 494 nucleotides. The declarant states that the nucleic acid sequence of Au Young matches PSCA at only 482 of its 494 bases (97%), and aligns with less than 50% of the 990 nucleotide PSCA sequence. Additionally, the declarant states that three gaps along the 494 nucleotide match range are created to achieve the alignment. Even with the gaps, compared to PSCA the SCAH-2 nucleic acid sequence was inaccurate at nine (9) positions.[77]

As stated in the attached Declaration, in view of the varied tissue sources and neoplastic statuses of the constituent elements of the Au Young nucleic acid assembly, the lack of any confirmatory cloning data, and lack of any information that constituent fragments were not contaminant or artifact, the scientific meaning, if any, that one of ordinary skill would attribute to the SCAH-2 assembly is exceedingly low. It is scientifically possible, and not in *any* way ruled out, that the sequence was merely a bioinformatic artifact, and does not exist at all. As set forth in the attached Declaration, in March 1997 it was not scientifically prudent to conclude that the SCAH-2 assembly actually existed, and one of ordinary skill in the art would not have made such a conclusion.

Much of the data in Au Young is extremely nonspecific. Hindsight must be avoided in order to prevent inadvertently attributing more meaning to the Au Young document than would have been done by one of ordinary skill in the art at the time.

2. **Assumption: That a protein was truly encoded by a/the SCAH-2 nucleic acid assembly, i.e., that it was a gene and not a pseudogene.**

   Fact: No SCAH-2 protein was ever documented by Au Young.

There is no data whatsoever in Au Young that a SCAH-2 protein actually existed or was present in any cell or sample . It is entirely possible that even if there were such a

[77] The SCAH-2 protein predicted by Au Young is labeled "Xaa" at our amino acid position 94 for unknown or unidentified.

nucleic acid sequence in the human genome, and even if Au Young appreciated that reading frame, that there was no actual coding gene at all and thus no corresponding protein. This possibility further undercuts any scientific credibility one of ordinary skill would attribute to Au Young concerning SCAH-2 protein.

As set forth in the attached Declaration, not only could a putative gene having a methionine codon followed by a downstream stop codon be a "false" gene because it encodes no RNA at all, a gene that encodes RNA can be a pseudogene because the RNA is degraded before it is translated into protein, the gene can be derived from reverse transcription of mRNA followed by reintegration into the genome with accompanying degradation and disablements[78], the gene can be derived from a duplication of an original gene followed by an initial coding disablement, potentially followed by further coding disablements.[79]

Given the inconclusive and oftentimes ambiguous information, one of ordinary skill would have looked on the Au Young disclosure regarding any SCAH-2 protein with scientific uncertainty. As stated in the attached Declaration, there is little scientific credibility in Au Young as to the existence in a cell or sample of the SCAH-2 assembly. Applicants submit that the only certainty as to any SCAH-2 protein of Au Young is provided in hindsight, and only by the knowledge provided in Applicants' disclosure. At most, Au Young *might* constitute an invitation for further experimentation in an attempt to arrive at information possessed of scientific credibility, e.g., information that meets the standards of written description and enablement.

3. **Assumption: That Au Young in a scientifically credible way appreciated the actual sequence of Applicants' PSCA protein.**

[78] see, e.g., Harrison et al., Genome Res 12: 272-280 (2002)
[79] see, e.g., Harrison et al., Genome Res 12: 272-280 (2002)

Fact: Au Young ambiguously referred to several amino acid sequences, some based on an open reading frame and others not; **it is not clear what SCAH-2 protein sequence is actually intended by the Au Young disclosure.**

Upon careful review of the Au Young disclosure as a whole, as required for a prior art evaluation, the existence and composition of any SCAH-2 amino acid sequence is even more unclear than is the nucleic acid sequence. Several amino acid sequences are set forth in Au Young any one, or even all, of which could have been what she intended as "a" or "the" SCAH-2 protein:

- As stated in the attached declaration, Figure 2 of Au Young provides an amino acid sequence that was fully deduced from Au-Young's entire nucleotide assembly. This sequence has 164 amino acids. No reading frame was appreciated in this figure. The alignment of PSCA is embedded within the Figure 2 SCAH-2 sequence. This SCAH-2 protein does not begin with a start methionine, as would prudently be done when no upstream stop codon was identified; the sequence continues past a stop codon as would prudently be done when there is uncertainty as to the accuracy of the sequence data.
- The protein in Au Young Figure 2 was designated SEQ ID 2 but was inaccurately set forth in Au-Young's Sequence Listing, it does not match the Figure.[80] This sequence begins with a methionine and has 123 amino acids. This discrepancy adds to confusion and merely invites further experimentation.
- Column 4, lines 62-65 states, "SCAH-2 has 27% identity to chicken stem cell antigen 2, is 123 amino acids long, and contains three potential glycosylation sites at N40, N83, and N96." This protein length conflicts with Figure 2, but the length does

[80] The *nucleic* acid sequence from Figure 2 does appear to match Au Young sequence listing SEQ ID NO 4.

correspond to Au-Young's SEQ ID 2. However, the three specified asparagines do not match either of these sequences, nor do they match any sequence Applicants could find in the Au Young document.

- If she meant SCAH-2 of Figure 2, none of the asparagines matched, and the "X" at position 96 is supposed to be asparagine and not unknown.
- If she meant the SCAH-2 protein in the Sequence Listing as SEQ ID 2, the asparagines at 40 and 83 match, position 94 is unknown, and position 96 is glycine not asparagine.
- If Au-Young's sentence from Column 4 is meant to refer to the chicken stem cell antigen-2 (Sca-2, Au-Young's SEQ ID 20) the length differs by 3 amino acids 123 vs. 126, contrary to Au Young, positions 40 and 83, are not aspargines but position 96 is aspargine.

Accordingly, Au Young ambiguously referred to at least six sequences, four permutations of which could be SCAH-2 assemblies. How was one of skill in the art know which are erroneous? In fact, it reflects hindsight to attribute the status of "erroneous" to the sequences, as this is properly viewed by one at the time as a research plan, an invitation for experimentation to confirm a gene and protein. As disclosed and without use of hindsight direction based on Applicants' PSCA, Au Young did not provide any particular protein sequence in a scientifically credible way described as SCAH-2, and certainly not the exact sequence of PSCA.

As stated in the attached Declaration, only hindsight motivates one to, amongst the various SCAH-2 related protein disclosure,

1) select only the 123 amino acid permutations;

2) decide what Au Young did not do about amino acid position 94, which she considered unknown,[81] turn to the nucleic acid sequence, use the two possible codons based on the "S" in the nucleic acid sequence, identify that these code for either alanine (GCC) or glycine (GGC);

3) select alanine and not glycine at position 94; and,

4) disregard Au Young at Column 4, lines 62-65 that SCAH-2 has asparagine at position 96 and use glycine instead.

Of note, Au Young did not do any of the above.[82] The disclosure is unclear about SCAH-2 amino acids and, by necessity, the composition of the corresponding codon. Furthermore, in March 1997, one would not have been motivated to choose amongst the disclosure of Au Young and arrive at the PSCA protein. Due to the lack of disclosure in Au Young, there was no reasonable expectation that any SCAH-2 protein existed at all, let alone a permutation that matches PSCA.

Hindsight cannot provide the certainty that Au Young herself lacked. Only hindsight, based on reviewing applicants' disclosure, resolves these ambiguities; this is not permitted in a proper patentability assessment of applicants' claims.

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[81] Applicants know of no principle that places authority on later individuals to answer why an earlier publication lacked information and/or resolve inconsistencies. Rather, Applicants submit that it is improper hindsight to fill in blanks after the fact. It is no more appropriate to attribute dominant meaning to the nucleic acid sequence and fill in the gap in the amino acid sequence with two alternatives (glycine or alanine), than it is to attribute dominant meaning to the unknown in the amino acid sequence and say that the corresponding codon was not known. Perhaps the "S" in the nucleic acid sequence was a typographical error; this is consistent with Au-Young's amino acid sequences.

[82] The Au Young codon at positions 286-288 of Figure 2 is GSC. The IUPAC definition for "S" is C or G.

4. **Assumption: That Au Young actually had a particular open reading frame for a SCAH-2 protein.**

Fact: It is ambiguous whether Au Young was certain of any open reading frame in the nucleic acid assembly. No amino acid sequence in any cell or sample was ever documented by Au Young.

Each of the various SCAH-2 proteins in the Au Young patent was a deduced sequence. Reviewing Au-Young's disclosure, as it would have been interpreted at the time it became available and without hindsight, it appears that either Au Young had not arrived at any SCAH-2 open reading frame at all, or if she did, was not sure she had a sufficiently valid nucleic acid sequence to make it credible. Once again, hindsight cannot provide the certainty that the Au Young disclosure lacked.

The SCAH-2 nucleic acid sequence and its corresponding protein are disclosed by Au Young as being set forth in Figure 2 (col. 5, lines 7-10). The sequence listing for this Figure properly corresponds for the nucleic acids (her SEQ ID 4), but improperly does not correspond for the amino acids (her SEQ ID 2). Inordinate meaning should not be inferred to a clearly improper sequence listing entry. Further, if Au Young at column 4, lines 62-65[83] truly indicates that the SCAH-2 protein is 123 amino acids, the specified asparagines do not match any sequence, and may have been meant to refer to the chicken sequence (her SEQ ID 20).

In making a prior art evaluation with Au Young it is not proper to selectively and differentially interpret parts of Au-Young's disclosure, e.g., on the basis of hindsight or merely because the Au Young sequence listing is easier to use for alignment purposes. Rather, the Au Young disclosure must be viewed in its entirety and as it would have been interpreted by one of ordinary skill in the art.

As set forth in the attached Declaration, that Au Young fully deduced the nucleic acid sequence of Figure 2 is consistent with the tentative status of Au-Young's data, and

[83] The sentence states, "SCAH-2 has 27% identity to chicken stem cell antigen 2, is 123 amino acids long, and contains three potential glycosylation sites at N40, N83, and N96."

the meaning of this amino acid information should not now be minimized. According to the attached Declaration, in March 1997 it was, and still is, scientifically prudent to deduce an entire nucleic acid when one is unclear about the extent of the sequence (e.g., if an upstream stop codon was not known) or the quality/veracity of the sequence is uncertain or low (e.g., a stop codon may be a sequence error).

The Au Young patent has the following: a figure that contains a sequence along with a SEQ ID identifier, a sequence listing filed simultaneously with the application, and the figure sequence and the corresponding SEQ ID differ. The information is confusing. Under these facts and as set forth in the attached Declaration, one of skill would attribute less meaning to the sequence listing.

As discussed in the attached Declaration, the following would be done upon reading Au Young and seeing that a sequence is referred to in a figure and a SEQ ID identifier is also provided. The figure would be reviewed. It would also be noted that patent authorities require a computer readable Sequence Listing. A scientist properly assumes that an applicant and relevant patent authorities would comply with the Sequence Listing requirements and would, in keeping with regulations, exactly set forth a sequence from a figure or specification in the Sequence Listing. If the scientist does ascertain that the sequence in the figure and the Sequence Listing do not match, between the figure sequence and the SEQ ID sequence, more weight is given to the figure. Moreover, the credibility of all of this information as definitive or as a scientifically credible disclosure is lowered. The lack of quality control makes one of skill call into question the apparent accuracy of all other information.

5. **Assumption: That any such multiple SCAH-2 proteins were scientifically credible so that would have been motivated to make antibodies against SCAH-2.**

Fact: No SCAH-2 protein was ever shown to be present in a cell or sample by Au Young **and no antibodies were raised by Au Young.**

Nothing in Au Young indicates that any antibodies to SCAH-2 were made or that any hybridomas that encode such antibodies were deposited. Furthermore, as stated in the attached Declaration, in a situation where one does not know:

- whether a nucleic acid assembly existed or was present in any cellor sample;
- if such a nucleic acid sequence did exist, what the open reading frame is;
- if such a nucleic acid did exist, whether it is merely a pseudogene,

one of ordinary skill could not and would not even begin to try to raise antibodies to some protein that may or may not be encoded. In this situation, raising of antibodies is not a method by which one would confirm existence of a putative protein. This was true in March 1997 and it is still true today. If one were motivated to conduct research on SCAH-2 due to Au Young, initial studies would attempt to confirm existence of the nucleic acid sequence, such as by gene cloning. This is confirmed in the attached Declaration.

Where the prior art is ambiguous, speculative, and lacks even a single working example, under patent law principles, there would be no utility possessed/appreciated by the art; no suitable description of a gene, a protein, protein expression or any antibodies to any such protein; and, no facts that enable one to use any gene, protein, protein expression or any antibodies to any such protein. There is no disclosure of elements foreclosing novelty of PSCA; and no disclosure of any elements combinable to yield PSCA obvious. As set forth in the attached Declaration, a protein not established to exist is not scientifically useful and not meaningfully described in a scientifically credible way—it was, as was said before, merely a wish, an invitation for further experimentation.

The reason for all this is simple: fundamental gene and protein discovery is lacking in Au Young.

Au Young as Disclosing a Disease Correlation Function for SCAH-2

Au Young did not establish to one of ordinary skill that, if a SCAH-2 protein were to exist, it had any scientifically credible meaning, let alone, a disease-associated meaning that would have mentioned one skilled in the art to make antibodiesagainst SCAH-2. Au Young states:[84]

> The present invention discloses novel human stem cell antigens (SCAH), characterized as having homology to Sca-2. Accordingly, the invention features substantially purified SCAH-1 and SCAH-2, encoded by the amino acid sequences of SEQ ID NO:1 and 2, respectively, and having characteristics of the LY-6 family of cysteine rich proteins which are expressed on the surface of lymphoid cells. ...
>
> The invention further provides diagnostic assays and kits for the detection of naturally occurring SCAH-1 or SCAH-2. It provides for the use of substantially purified SCAH-1 or SCAH-2 as a positive control and to produce anti-SCAH-1 or SCAH-2 antibodies which can be used to quantitate the amount of SCAH proteins in human body fluids or biopsied tissues. ...
>
> Substantially purified SCAH-1 or SCAH-2 or their fragments may be useful as pharmaceutical compositions. For example, they may be used to inhibit or reverse the development of tumors.

[84] (Au Young, '391, column 2, lines 12-18, 43-49, 52-55)

These passages must be interpreted in light of the overall disclosure of Au Young and cannot be taken out of context and viewed as definitive isolated assertions.

In related prosecution[85] the USPTO alleged that the Au Young sentence, "[t]he nucleic acid sequence encoding a portion of the novel stem cell antigen homolog-2 (designated in lower case, scah-2) was present in tissues removed from bladder tumor and uterus"[86] teaches treating a patient having bladder tumor, using antibodies against SCAH-2. Applicants disagree: this assertion is not scientifically credible, and would not have been made based on Au Young at the proper time frame.

As set forth in the attached Declaration, one could not in March 1997, or even now, draw scientifically credible conclusions from Column 4, lines 27-30 of Au Young. Au Young's detection of portions of SCAH-2 cDNA in bladder and uterus tissues does not describe or suggest correlation of SCAH-2 with cancer in those tissues with any scientific credibility.[87]

The Patent Office's reasoning assumes that every nucleic acid assembly that might possibly encode a protein, does encode a protein; if multiple variant proteins could be encoded, they all are; and, that protein is expressed in every tissue from which ESTs were derived. This is not scientifically credible. Au Young does not teach that SCAH-2 protein was observed or was present in bladder tumor. Au Young does not teach that SCAH-2 gene or protein even exists in a cell or sample.

Of particular note, the fact that the source tissues were from two states of neoplasia teaches away from an idea that the SCAH-2 assembly correlated with a

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[85] U.S.S.N. 09/963,620, Paper 9, page 22. *Note, the USPTO statement referred to lines 29-30 only of a sentence that extends from lines 27-30. If that portion is taken out of context, it appears to say something quite different that the sentence does properly viewed as a whole.*

[86] Au Young '391 patent, column 4, lines 27-30

[87] The basis for this reasoning, if applied to uterine tissue, seems to be that, upon locating a particular partial gene sequence in a tissue, it becomes scientifically credible that there is cancer in that tissue. In contrast to such an assertion, however, it is a well-recognized characteristic of neoplastic cells that they have deviant characteristics compared to respective normal, non-neoplastic cells. This is particularly true of malignant, cancerous neoplasms.

particular disease state such as cancer. Au Young merely states that "portions" of the nucleic acid of SCAH-2 were present in tissues removed from bladder tumor. Other than using portions of SCAH-2 cDNA from bladder and uterus tissue libraries, Au Young provides no characterization or expression data on SCAH-2.[88]

Moreover, the only mention of cancer treatment in Au Young occurs in the context of polynucleotides, not protein, and generically relates to all polynucleotides in the document, and is a laundry list of potential cancers:[89]

> Therapeutics
>
> The polynucleotides disclosed herein may be useful in the treatment of conditions associated with the tissues used to construct the cDNA libraries (shown in the Sequence ID Listing) which contained partial scah sequences. These include, but are not limited to, conditions such as leukemias and cancers of the bladder, breast, lung, ovary, prostate, and uterus.

The quoted paragraph is about *both* SCAH-1 and SCAH-2 . This broad listing of potential cancers certainly does not establish to one of ordinary skill or make obvious to them that these cancers are correlated in any way with either SCAH-1, SCAH-2, or both. If this were to be evaluated under an obviousness scenario it fails, as there is no reasonable expectation of success that SCAH-1 or SCAH-2 would be found in those randomly assorted tissues or are related to any disease condition.

If the SCAH-2 assemblies were found to exist in a cell or sample, and actually encoded a protein, but without any neoplasia-associated characterization and/or tissue localization data, as stated in the attached declaration, one of the most plausible

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[88] This is precisely a context in which said "portion" may not be expressed in the asserted protein at all, but in a variant thereof.

[89] Au Young '391, column 18, lines 15-21

conclusions is that it was merely a housekeeping-type gene.[90] Many of the about 30,000 genes in a cell are housekeeping genes. But it would not have been plausible to correlate SCAH- with cancer in any way.

A conclusion that SCAH-2 is a ubiquitous gene is particularly plausible for SCAH-2, because SCAH-2 was derived from both normal and tumor sources and from two different tissues. Without more data, there was no reason to implicate SCAH-2 in cancer based on the disclosure of Au Young.[91]

These principles are exemplified for the tissues from which fragments were derived to construct the SCAH-2 nucleic acid assembly: normal uterus and bladder tumor. It is not scientifically credible to infer cancer tissue expression based on the cDNA fragments from normal uterus. The converse of this argument applies to the bladder tumor component of the nucleic acid assembly. Just because cDNA fragment was derived from a tumor cell does not mean it is not in a normal cell. There is not enough scientific information in the mere existence of the nucleic acid assembly in Au Young to determine any particular malignant or normal expression pattern.

Moreover, even if any function or disease association had been documented for SCAH-2, the lack of actual expression data is a serious impediment to the obviousness of any antibodies. Even if the protein exists, it could be expressed at such low levels or turned over so rapidly that raising of antibodies to it is not scientifically relevant, i.e., not an obvious thing to do.

Further, as Au Young only hypothesized and never expressed SCAH-2, no tissue localization/distribution data was possible from the Au Young disclosure. Au Young did not know whether the SCAH-2 assemblies were present in any cells or samples; were

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[90] For example, it might exist in cancer tissues or it might not exist in cancer tissues; if it exists in cancer tissues, it might be over expressed in cancer tissues compared to normal tissues or it may not be over expressed in cancer tissues compared to normal tissues.

[91] Moreover, any attempt to correlate SCAH-2 with any other tissue, such as a tissue mentioned at Au Young column 18 is totally specious. Those tissues are components of other nucleic acid assembly sequences of ESTs in the document.

ubiquitous; were expressed, and if expressed whether such expression was found in only select cells such as malignant cells; or, overexpressed on any particular tissue.

Further, without localization data, there was no way to know whether SCAH-2 were expressed in vital organs such as the heart or lung. Cytotoxic targeting of vital organs is certainly to be avoided on scientific and ethical bases. For example, one would not even consider a treatment without knowing if the treatment itself could kill the patient.

At most one could say that SCAH-2 transcript fragments (but not the entire SCAH-2 assemblies) were found in both normal and tumor cells, but without any actual expression data (e.g., upregulation in tumor versus normal, vital organ expression, etc.) Au Young teaches away from using SCAH-2 as a target for cancer therapy. Fundamental discovery regarding disease correlation is lacking.

In the absence of any specific correlation of SCAH-2 with malignant tissues and not with vital organ tissues, it would be scientifically contraindicated and ethically improper to target cells in humans that express SCAH-2. Thus, Au Young does not anticipate or make obvious such use.

Au Young as Disclosing Stem Cell Antigen or Ly-6 Function for SCAH-2

As to any functional aspect of SCAH-2, Au Young did not establish to one of ordinary skill that a SCAH-2 protein, if it were to exist, had any particular functional meaning that would have made the raising of antibodies relevant. There is no function-related disclosure, such as motif or alignment identity, that is compelling so as to indicate, that protein may truly exist in a cell.

Au Young speculated as to two functions to SCAH-2; neither of these would have been reasonably expected to be true let alone definitive as of the filing date of the present invention such that antibodies would be raised.

First, Au Young speculated/concludes that the SCAH-2 protein serves as a stem cell antigen. That is the basis for the name *S*tem *C*ell *A*ntigen *H*omolog (SCAH).[92] This speculation/conclusion is based on a low identity alignment to a non-mammalian, chicken sequence.[93] The identity was only 27%; moreover the 27 % was not localized in a manner that would cause one to contemplate a particular, conserved sequence associated with some function, i.e., a functional domain or motif but was distributed throughout. As set forth in the attached Declaration, the comparison of the chicken sequence to SCAH-2 as the last two rows of Au Young '136, Figure 3 does not indicate to one of ordinary skill that SCAH-2 has stem cell antigen function. One of ordinary skill would not have attributed definitive stem cell antigen function to SCAH-2 based on this data. At most, Au Young may have provided an invitation for further research to ultimately determine whether a putative protein exists and functions as a stem cell antigen. Nevertheless, fundamental gene and protein discovery needed to occur before any antibodies would be raised.

Second, Au Young speculated that the SCAH-2 protein might function like a Ly-6 molecule.[94] Scientifically, Au Young did not established that SCAH-2 is in fact associated with Ly-6, neither is there a reasonable expectation that it is.

However, even if SCAH-2 were a member of the Ly 6 family, there is no common function associated with the disparate members of the Ly-6 family. For example, some Ly-6 molecules are cell surface molecules that are involved in signal transduction,[95] or cell adhesion.[96] Others are secreted molecules that are involved with neurotoxicity or cytotoxicity,[97] or diseases unrelated to cancer.[98]

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[92] Au Young '391 patent, column 4, lines 16-17

[93] Au Young '391 patent, column 4, lines 62-65

[94] Au Young '391 patent at column 2, lines 16-19; column 13, lines 41-47; column 16, lines 14-17; column 28, lines 13-21

[95] e.g., Gumley, et al., 1995, Immunol and Cell Biol, 73(4):277-96

[96] e.g., Brakenhoff, et al., 1995, J Cell Biol, 129(6):1677-1689

[97] e.g., Tsetlin, 1999, Eur J Biochem, 264:281-286

[98] e.g., Fischer et al., 2001, Human Mol Genet, 10(8):875-880

Given the broad range of potential Ly-6 functions, it would not have been credible to one of ordinary skill to extrapolate a particular scientifically credible function of SCAH-2 based on the other members of the Ly-6 family. Once again, at most, Au Young may provide an invitation for further research to ultimately determine whether it functions like Ly-6, and if so identify which of the many disparate functions of the numerous Ly-6 family members SCAH-2 possesses. Even if there were an invitation for experimentation, such experimentation would not have included the raising of antibodies before a protein is credibly determined to actually occur in nature, again fundamental gene and protein discovery was lacking. Fundamental gene and protein discovery needed to occur before any antibodies could be raised.

The Spitler Reference

Spitler (U.S. Patent No. 5,738,867) teaches methods and compositions employing liposome compositions encapsulating or conjugated to Tumor Associated Antigens (TAAs), or anti-idiotypic monoclonal antibodies to TAAs. In specific embodiments, Spitler discloses liposome compositions encapsulating the TAA CO-029, which is associated with tumors of the gastrointestinal tract, colorectum, and pancreas. Spitler also teaches liposome compositions encapsulating the TAA GA733-2, which is associated with tumors of the gastrointestinal tract, prostate, cervix, ovary, bladder, lung, breast, colorectum, and pancreas. Additionally, Spitler discloses methods and compositions for conjugating TAAs to liposomes.

Contrary to the Office's assertion, Spitler does not teach that the TAA-liposome compositions are capable of eliciting an immune or anti-tumor response in a subject. Although Spitler mentions immunizing mice and humans in Examples X – XII, these are prophetic examples and there is no data or indication that any of these TAA-liposome compositions actually induce any immune response, let alone an anti-tumor response.

There is no Suggestion or Motivation to Combine Spitler with Au Young

Spitler does not teach the use of TAA in an immune response. Accordingly, the combination of Spitler and Au Young, where one replaces TAA with SCAH to induce an immune response, cannot be obvious.

However, even assuming arguendo that Spitler does teach TAA in an immune response, there is no suggestion or motivation in Spitler or in Au Young, to substitute the multiple SCAH assemblies, let alone the multiple SCAH-2 assemblies, for TAA.

Additionally Au-Young failed to teach any use for SCAH-2 leading to no motivation to use it to regulate an immune response.

The caselaw is clear. There is no motivation to substitute one compound for another unless the two compounds share a common feature or can be considered as equivalents (In re Lalu and Foulletier 747 F.2D 703, 223 U.S.P.Q. 1257 (Fed. Cir. 1984). Since Au-Young did not know whether the SCAH-2 assemblies existed in any cell or sample (for the reasons disclosed above) or the function of SCAH-2, based on Au-Young one could not have known whether SCAH-2 could be an equivalent of TAA.

Moreover, the analysis as to obviousness does not stop at whether it would have been obvious to replace TAA with SCAH-2. The obviousness analysis continues as to whether it would have been obvious to replace any of the SCAH assemblies, in particular the multiple SCAH-2 assemblies, with the PSCA of SEQ ID NO. 2 so as to obtain the claimed methods. We believe not. As discussed above, there was no known use for any of the multiple SCAH-2 assemblies. Therefore, one would not have replaced any of the multiple SCAH-2 assemblies with the PSCA of the invention.

Hindsight reconstruction of the claimed invention.

It is a well-settled principle of patent law that motivation to combine must come from the prior art to render a claim obvious. No motivation for using SCAH-2 in place of TAA is found in Au Young for reasons discussed above.

Additionally, no motivation for using PSCA in place of TAA or SCAH-2 is found in either Spitler or Au Young.

It is only upon reviewing applicants' invention that one skilled in the art would be motivated to substitute SCAH-2 for PSCA or even PSCA for TAA. The Patent Office's position at best, is one involving an obvious to try standard.

As noted by the Federal Circuit [99] an obvious to try context is one where one needs to:

1) to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful.

In the instant rejection, one is substituting essentially any compound of the art from Au Young for the TAA of Spitler in order to induce an immune response as claimed. However, as stated above, there is no rational basis for the substitution if the SCAH-2 protein of Au Young is not known to exist.

Accordingly, as Au Young does not provide disclosure or enablement of elements of the present invention, it is not a sufficient basis for obviousness rejection of the pending claims. These defects are not cured by the secondary references. Accordingly, withdrawal of the Section 103 rejections is requested.

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[99] In re O'Farrell, 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988)

Rejection Based on the Combination of Spitler and Billing-Medel

In paragraph 14 of the Office Action, the Examiner further rejected claim 73 as being unpatentable over Billing-Medel in view of Spitler et al. Applicants respectfully disagree. However, in order to further the prosecution of the subject application, applicants have removed reference to bladder cancer from the claim.

CONCLUSION

Au Young does not describe the claimed invention in terms that will "enable any person skilled in the art ... to make and use" the invention. Au Young does not enable what she did not describe. At most, its description will invite a person of ordinary skill in the art to *attempt to discover* if they can practice the claimed invention. That is not enough.

In order to be patent defeating, the prior art must describe the subject matter to be patented with such clarity that a person of ordinary skill in the field is placed in possession of it. The Au Young patent does not do that. Instead, Au-Young's disclosure about SCAH-2 insofar as its sufficiency for purposes of the description is concerned, creates a situation analogous to the one addressed by Lilly, where the court stated that "[t]he description requirement of the patent statute requires a description of an invention, not an indication of a result that one might achieve if one made that invention."[100]

"Tossing out the mere germ of an idea does not constitute enabling disclosure."[101] Furthermore the Supreme Court has stated, "a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion."[102] Thus, while the need for some experimentation is by no means necessarily fatal, "reasonable detail must be provided in order to enable members of the public to understand and carry out the

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[100] 119 F.3d at 1568

[101] Genentech, 108 F.3d at 1366

[102] Brenner v. Manson, 383 U.S. 519, 536, 86 S.Ct. 1033, 16 L.Ed.2d 69 (1966) (stated in context of the utility requirement)

invention."[103] Such detail is lacking in Au Young. At most Au Young constitutes non-enabled, germinal ideas about SCAH-2.

The Federal Circuit in Fiers made the following observation about "[t]he difficulty that would arise if we were to hold that a conception occurs when one has only the idea of a compound, defining it by its hoped-for function"[104]:

> would-be inventors would file patent applications before they had made their inventions and before they could describe them. That is not consistent with the statute or the policy behind the statute, which is to promote disclosure of inventions, not of research plans. While one does not need to have carried out one's invention before filing a patent application, one does need to be able to describe that invention with particularity.

That concern is well illustrated by Au Young, and its use as a prior art reference.

In view of the present submission, Applicants contend that the subject application is in condition for allowance. Accordingly, issuance of a notice of allowance is requested.

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[103] Id.

[104] Fiers, 984 F.2d at 1169.

No fee is due in connection with this Preliminary Amendment. However, if a fee is deemed necessary, applicants authorize the Patent Office to charge the fee to the Deposit Account No. 50-0306.

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METHODS FOR INDUCING AN IMMUNE RESPONSE TO CANCERS EXPRESSING PSCA~~PSCA: PROSTATE STEM CELL ANTIGEN AND USES THEREOF~~

This application is a divisional application of U.S. Serial No. 09/564,329 filed May 3, 2000, now issued U.S. Patent No. 6,541,212, issued on April 1, 2003, which is a continuation-in-part (CIP) of U.S. Serial No. 09/359,326 filed July 20, 1999, now abandoned, which is a CIP of U.S. Serial No. 09/318,503 filed May 25, 1999, now issued U.S. Patent No. 6,261,791, issued on July 17, 2001, which is a CIP of U.S. Serial No. 09/251,835 filed February 17, 1999, now issued U.S. Patent No. 6,261,789, issued on July 17, 2001, which is a CIP of U.S. Serial No. 09/203,939 filed December 2, 1998, now issued U.S. Patent No. 6,258,939, issued on July 10, 2001, which is a CIP of U.S. Serial No. 09/038,261 filed March 10, 1998, now issued U.S. Patent No. 6,267,960, issued July 31, 2001, claiming the priority of provisional applications, U.S. Serial No. 60/228,816 filed March 10, 1997; U.S. Serial No. 60/071,141 filed January 12, 1998 and U.S. Serial No. 60/074,675 filed February 13, 1998. This application which is a divisional application of U.S. Serial No. 09/564,329 filed May 3, 2000, now issued U.S. Patent No. 6,541,212, issued on April 1, 2003, further claims the benefit of the filing dates of U.S. Serial No. 60/113,230 filed December 21, 1998, U.S. Serial No. 60/120,536 filed February 17, 1999 and U.S. Serial No. 60/124,658 filed March 16, 1999.~~This application is a Divisional application of U.S. Serial No. 09/564,329, filed May 3, 2000, which is a continuation-in-part (CIP) of U.S. Serial No. 09/359,326, filed July 20, 1999, which is a CIP of U.S. Patent No. 6,261,791, issued July 17, 2001, based on U.S. Serial No. 09/318,503, filed May 25, 1999, which is a CIP of U.S. Patent No. 6,261,789, issued July 17, 2001, based on U.S. Serial No. 09/251,835, filed February 17, 1999, which is a CIP of U.S. Patent No. 6,258,939, issued July 10, 2001, based on U.S. Serial No. 09/203,939, filed December 2, 1998, which is a CIP of U.S. Patent No. 6,267,960, issued July 31, 2001, based on U.S. Serial No. 09/038,261, filed March 10, 1998[,]; claiming the priority of provisional applications, U.S. Serial No. 60/228,816, filed March 10, 1997[,]; U. S. Serial No. 60/071,141, filed January 12, 1998 and; U. S. Serial No. 60/ 074,675, filed February 13, 1998. This application further claims the benefit of the filing dates of U.S. Serial Nos. 60/124,658, filed March 16, 1999; 60/120,536 filed February 17, 1999; and~~

60/113,230 filed December 21, 1998. The contents of all of the foregoing applications are incorporated by reference into the present application.

Throughout this application, various publications are referenced within parentheses. The disclosures of these publications are hereby incorporated by reference herein in their entireties.

BACKGROUND OF THE INVENTION

Cancer is the second leading cause of human death next to coronary disease. Worldwide, millions of people die from cancer every year. In the United States alone, cancer cause the death of well over a half-million people each year, with some 1.4 million new cases diagnosed per year. While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the next century, cancer is predicted to become the leading cause of death.

Worldwide, several cancers stand out as the leading killers. In particular, carcinomas of the lung, prostate, breast, colon, pancreas, and ovary represent the leading causes of cancer death. These and virtually all other carcinomas share a common lethal feature. With very few exceptions, metastatic disease from a carcinoma is fatal. Moreover, even for those cancer patients that initially survive their primary cancers, common experience has shown that their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the potential for recurrence or treatment failure. Many cancer patients experience significant physical debilitations following treatment.

Generally speaking, the fundamental problem in the management of the deadliest cancers is the lack of effective and non-toxic systemic therapies. Molecular medicine, still very much in its infancy, promises to redefine the ways in which these cancers are managed. Unquestionably, there is an intensive worldwide effort aimed at the development of novel molecular approaches to cancer diagnosis and treatment. For example, there is a great

interest in identifying truly tumor-specific genes and proteins that could be used as diagnostic and prognostic markers and/or therapeutic targets or agents. Research efforts in these areas are encouraging, and the increasing availability of useful molecular technologies has accelerated the acquisition of meaningful knowledge about cancer. Nevertheless, progress is slow and generally uneven.

Recently, there has been a particularly strong interest in identifying cell surface tumor-specific antigens which might be useful as targets for various immunotherapeutic or small molecule treatment strategies. A large number of such cell-surface antigens have been reported, and some have proven to be reliably associated with one or more cancers. Much attention has been focused on the development of novel therapeutic strategies which target these antigens. However, few truly effective immunological cancer treatments have resulted.

The use of monoclonal antibodies to tumor-specific or over-expressed antigens in the treatment of solid cancers is instructive. Although antibody therapy has been well researched for some 20 years, only very recently have corresponding pharmaceuticals materialized. One example is the humanized anti-HER2/neu monoclonal antibody, Herceptin, recently approved for use in the treatment of metastatic breast cancers overexpressing the HER2/neu receptor. Another is the human/mouse chimeric anti-CD20/B cell lymphoma antibody, Rituxan, approved for the treatment of non-Hodgkin's lymphoma. Several other antibodies are being evaluated for the treatment of cancer in clinical trials or in pre-clinical research, including a chimeric and a fully human IgG2 monoclonal antibody specific for the epidermal growth factor receptor (Slovin et al., 1997, Proc. Am. Soc. Clin. Oncol. 16:311; Falcey et al., 1997, Proc. Am. Soc. Clin. Oncol. 16:383; Yang et al., 1999, Cancer Res. 59: 1236). Evidently, antibody therapy is finally emerging from a long embryonic phase. Nevertheless, there is still a very great need for new, more-specific tumor antigens for the application of antibody and other biological therapies. In addition, there is a corresponding need for tumor antigens which may be useful as markers for antibody-based diagnostic and imaging methods, hopefully leading to the development of earlier diagnosis and greater prognostic precision.

As discussed below, the management of prostate cancer serves as a good example of the limited extent to which molecular biology has translated into real progress in the clinic. With limited exceptions, the situation is more or less the same for the other major carcinomas mentioned above.

Worldwide, prostate cancer is the fourth most prevalent cancer in men. In North America and Northern Europe, it is by far the most common male cancer and is the second leading cause of cancer death in men. In the United States alone, well over 40,000 men die annually of this disease, second only to lung cancer. Despite the magnitude of these figures, there is still no effective treatment for metastatic prostate cancer. Surgical prostatectomy, radiation therapy, hormone ablation therapy, and chemotherapy remain as the main treatment modalities. Unfortunately, these treatments are clearly ineffective for many. Moreover, these treatments are often associated with significant undesirable consequences.

On the diagnostic front, the serum PSA assay has been a very useful tool. Nevertheless, the specificity and general utility of PSA is widely regarded as lacking in several respects. Neither PSA testing, nor any other test nor biological marker has been proven capable of reliably identifying early-stage disease. Similarly, there is no marker available for predicting the emergence of the typically fatal metastatic stage of the disease. Diagnosis of metastatic prostate cancer is achieved by open surgical or laparoscopic pelvic lymphadenectomy, whole body radionuclide scans, skeletal radiography, and/or bone lesion biopsy analysis. Clearly, better imaging and other less invasive diagnostic methods offer the promise of easing the difficulty those procedures place on a patient, as well as improving therapeutic options. However, until there are prostate tumor markers capable of reliably identifying early-stage disease, predicting susceptibility to metastasis, and precisely imaging tumors, the management of prostate cancer will continue to be extremely difficult. Accordingly, more specific molecular tumor markers are clearly needed in the management of prostate cancer.

There are some known markers which are expressed predominantly in prostate, such as prostate specific membrane antigen (PSM), a hydrolase with 85% identity to a rat neuropeptidase (Carter et al., 1996, Proc. Natl. Acad. Sci. USA 93: 749; Bzdega et al., 1997, J. Neurochem. 69: 2270). However, the expression of PSM in small intestine and brain (Israeli et al., 1994, Cancer Res. 54: 1807), as well its potential role in neuropeptide catabolism in brain, raises concern of potential neurotoxicity with anti-PSM therapies. Preliminary results using an Indium-111 labeled, anti-PSM monoclonal antibody to image recurrent prostate cancer show some promise (Sodee et al., 1996, Clin Nuc Med 21: 759-766). More recently identified prostate cancer markers include PCTA-1 (Su et al., 1996, Proc. Natl. Acad. Sci. USA 93: 7252). PCTA-1, a novel galectin, is largely secreted into the media of expressing cells and may hold promise as a diagnostic serum marker for prostate cancer (Su et al., 1996). Vaccines for prostate cancer are also being actively explored with a variety of antigens, including PSM and PSA.

SUMMARY OF THE INVENTION

The invention provides a novel prostate cell-surface antigen, designated Prostate Stem Cell Antigen (PSCA), which is widely over-expressed across all stages of prostate cancer, including high grade prostatic intraepithelial neoplasia (PIN), androgen-dependent and androgen-independent prostate tumors. The PSCA gene shows 30% homology to stem cell antigen-2 (SCA-2), a member of the Thy-1/Ly-6 family of glycosylphosphatidylinositol (GPI)-anchored cell surface antigens, and encodes a 123 amino acid protein with an amino-terminal signal sequence, a carboxy-terminal GPI-anchoring sequence, and multiple N-glycosylation sites. PSCA mRNA expression is highly upregulated in both androgen dependent and androgen independent prostate cancer xenografts. In situ mRNA analysis localizes PSCA expression to the basal cell epithelium, the putative stem cell compartment of the prostate. Flow cytometric analysis demonstrates that PSCA is expressed predominantly on the cell surface and is anchored by a GPI linkage. Fluorescent in situ hybridization analysis localizes the PSCA gene to chromosome 8q24.2, a region of allelic gain in more than 80% of prostate cancers.

PSCA may be an optimal therapeutic target in view of its cell surface location, greatly upregulated expression in certain types of cancer such as prostate cancer cells. In this regard, the invention provides antibodies capable of binding to PSCA which can be used therapeutically to destroy or inhibit the growth of such cancer cells, or to block PSCA activity. In addition, PSCA proteins and PSCA-encoding nucleic acid molecules may be used in various immunotherapeutic methods to promote immune-mediated destruction or growth inhibition of tumors expressing PSCA.

PSCA also may represent an ideal prostate cancer marker, which can be used to discriminate between malignant prostate cancers, normal prostate glands and non-malignant neoplasias. For example, PSCA is expressed at very high levels in prostate cancer in relation to benign prostatic hyperplasia (BPH). In contrast, the widely used prostate cancer marker PSA is expressed at high levels in both normal prostate and BPH, but at lower levels in prostate cancer, rendering PSA expression useless for distinguishing malignant prostate cancer from BPH or normal glands. Because PSCA expression is essentially the reverse of PSA expression, analysis of PSCA expression can be employed to distinguish prostate cancer from non-malignant conditions.

The genes encoding both human and murine PSCA have been isolated and their coding sequences elucidated and provided herein. Also provided are the amino acid sequences of both human and murine PSCA. The invention further provides various diagnostic assays for the detection, monitoring, and prognosis of prostate cancer, including nucleic acid-based and immunological assays. PSCA-specific monoclonal and polyclonal antibodies and immunotherapeutic and other therapeutic methods of treating prostate cancer are also provided. These and other aspects of the invention are further described below.

BRIEF DESCRIPTION OF THE FIGURES

[**FIG. 1.** Nucleotide (A) and translated amino acid (B) sequences of a cDNA encoding human PSCA (ATCC Designation 209612).] **FIG 1A.** Nucleotide sequence (SEQ ID NO:1, ATCC Designation 209612) of a cDNA encoding human PSCA.

FIG. 1B. Translated amino acid sequence (SEQ ID NO:2) of human PSCA.

[**FIG. 2.** Nucleotide sequence of a cDNA encoding murine PSCA homologue.] **FIG. 2.** Nucleotide sequence (SEQ ID NO:3), of a murine cDNA PSCA homologue and the translated amino acid sequence (SEQ ID NO:4) of murine PSCA.

FIG. 3. Alignment of amino acid sequences of human PSCA (SEQ ID NO:5), murine PSCA (SEQ ID NO:6), and human stem cell antigen-2 (hSCA-2) (SEQ ID NO:7). Shaded regions highlight conserved amino acids. Conserved cysteines are indicated by bold lettering. Four predicted N-glycosylation sites in PSCA are indicated by asterisks. The underlined amino acids at the beginning and end of the protein represent N terminal hydrophobic signal sequences and C terminal GPI-anchoring sequences, respectively.

FIG. 4. Hydrophobicity plot of human PSCA.

FIG. 5. Chou-Fassman analysis of human PSCA.

FIG. 6. A western blot showing that monoclonal antibody 1G8 binds LAPC9 (PSCA positive control) and a transitional cell carcinoma (bladder carcinoma) designated bladder (Rob).

[**FIG. 7.** Restricted Expression of PSCA mRNA in normal and cancerous tissues. A: RT-PCR analysis of PSCA expression in normal human tissues demonstrating high expression in prostate, placenta, and tonsils. 1ng of reverse-transcribed first strand cDNA (Clontech, Palo Alto, CA) from the indicated tissues was amplified with PSCA gene specific primers.

Data shown are from 30 cycles of amplification. B: RT-PCR analysis of PSCA expression demonstrating high level in prostate cancer xenografts and normal tissue. 5ng of reverse-transcribed cDNA from the indicated tissues were amplified with PSCA gene specific primers. Amplification with β-actin gene specific primers demonstrate normalization of the first strand cDNA of the various samples. Data shown are from 25 cycles of amplification. AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.] **FIG. 7A.** Restricted Expression of PSCA mRNA in normal and cancerous tissues. RT-PCR analysis of PSCA expression in normal human tissues demonstrating high expression in prostate, placenta, and tonsils. 1ng of reverse-transcribed first strand cDNA (Clontech, Palo Alto, CA) from the indicated tissues was amplified with PSCA gene specific primers. Data shown are from 30 cycles of amplification.

FIG. 7B. Restricted Expression of PSCA mRNA in normal and cancerous tissues. RT-PCR analysis of PSCA expression demonstrating high level in prostate cancer xenografts and normal tissue. 5 ng of reverse-transcribed cDNA from the indicated tissues was amplified with PSCA gene specific primers. Amplification with β-actin gene specific primers demonstrate normalization of the first strand cDNA of the various samples. Data shown are from 25 cycles of amplification. AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

[**FIG. 8.** Schematic representation of human PSCA, murine PSCA, and human Thy-1/Ly-6 gene structures.] **FIG. 8A.** Schematic representation of human Thy-1/Ly-6 gene structures.

FIG. 8B. Schematic representation of murine PSCA gene structure.

FIG. 8C. Schematic representation of human PSCA gene structure.

[**FIG. 9.** Northern blot analysis of PSCA RNA expression. **A:** Total RNA from normal prostate and LAPC-4 androgen dependent (AD) and independent (AI) prostate cancer xenografts were analyzed using PSCA or PSA specific probes. Equivalent RNA loading

and RNA integrity were demonstrated separately by ethidium staining for 18S and 28S RNA. **B:** Human multiple tissue Northern blot analysis of PSCA RNA. The filter was obtained from Clontech (Palo Alto, CA) and contains 2ug of polyA RNA in each lane.]

FIG. 9A. Northern blot analysis of PSCA expression. Total RNA from normal prostate and LAPC-4 androgen dependent (AD) and independent (AI) prostate cancer xenografts were analyzed using PSCA or PSA specific probes. Equivalent RNA loading and RNA integrity were demonstrated separately by ethidium staining for 18S and 28S RNA.

FIG. 9B. Northern blot analysis of PSCA expression. Human multiple tissue Northern blot analysis of PSCA. The filter was obtained from Clontech (Palo Alto, CA) and contains 2ug of polyA RNA in each lane.

[**FIG. 10.** Northern blot comparison of PSCA, PSMA, and PSA RNA expression in prostate cancer xenografts and tumor cell lines. PSCA and PSMA demonstrate high level prostate cancer specific gene expression. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSCA, PSMA, and PSA cDNA fragments. Shown are 4 hour and 72 hour autoradiographic exposures of the membrane and the ethidium bromide gel demonstrating equivalent loading of samples. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.] **FIG. 10A.** Northern blot analysis of PSCA expression in prostate cancer xenografts and tumor cell lines. PSCA demonstrates high level prostate cancer specific gene expression. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSCA cDNA fragments. Shown are 4 hour and 72 hour autoradiographic exposures of the membrane. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

FIG. 10B. Northern blot analysis of PSM expression in prostate cancer xenografts and tumor cell lines. PSM demonstrates high level prostate cancer specific gene expression.

10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSM cDNA fragments. Shown are 4 hour and 72 hour autoradiographic exposures of the membrane. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

FIG. 10C. Northern blot analysis of PSA expression in prostate cancer xenografts and tumor cell lines. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSA cDNA fragments. Shown are 4 hour and 72 hour autoradiographic exposures of the membrane and the ethidium bromide gel demonstrating equivalent loading of samples. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

[**FIG. 11.** In situ hybridization with antisense riboprobe for human PSCA RNA on normal and malignant prostate specimens. **A:** PSCA RNA is expressed by a subset of basal cells within the basal cell epithelium (black arrows), but not by the terminally differentiated secretory cells lining the prostatic ducts (400X magnification). **B:** PSCA RNA is expressed strongly by a high grade prostatic intraepithelial neoplasia (PIN) (black arrow) and by invasive prostate cancer glands (yellow arrows), but is not detectable in normal epithelium (green arrow) at 40X magnification. **C:** Strong expression of PSCA RNA in a case of high grade carcinoma (200X magnification).] **FIG. 11A.** In situ hybridization with antisense riboprobe for human PSCA on normal prostate specimens. PSCA is expressed by a subset of basal cells within the basal cell epithelium (black arrows), but not by the terminally differentiated secretory cells lining the prostatic ducts (400X magnification).

FIG. 11B. In situ hybridization with antisense riboprobe for human PSCA on normal and malignant prostate specimens. PSCA is expressed strongly by a high grade prostatic intraepithelial neoplasia (PIN) (black arrow) and by invasive prostate cancer glands

(yellow arrows), but is not detectable in normal epithelium (green arrow) at 40X magnification.

FIG. 11C. In situ hybridization with antisense riboprobe for human PSCA on malignant prostate specimens. Strong expression of PSCA in a case of high grade carcinoma (200X magnification).

[FIG. 12. Biochemical analysis of PSCA protein. A: PSCA protein was immunoprecipitated from 293T cells transiently transfected with a PSCA construct and then digested with either N-glycosidase F or O-glycosidase, as described in Materials and Methods. B: PSCA protein was immunoprecipitated from 293T transfected cells, as well as from conditioned media of these cells . Cell-associated PSCA migrates higher than secreted or shed PSCA on a 15% polyacrylamide gel. C:FACS analysis of mock-transfected 293T cells, PSCA-transfected 293T cells and LAPC-4 prostate cancer xenograft cells using an affinity purified polyclonal anti-PSCA antibody. Cells were not permeabilized in order to detect only surface expression. The y axis represents relative cell number and the x axis represents fluorescent staining intensity on a logarithmic scale.] FIG. 12A. Biochemical analysis of PSCA. PSCA was immunoprecipitated from 293T cells transiently transfected with a PSCA construct and then digested with either N-glycosidase F or O-glycosidase, as described in Materials and Methods.

FIG. 12B. Biochemical analysis of PSCA. PSCA was immunoprecipitated from 293T transfected cells, as well as from conditioned media of these cells . Cell-associated PSCA migrates higher than secreted or shed PSCA on a 15% polyacrylamide gel.

FIG 12C. Biochemical analysis of PSCA. FACS analysis of mock-transfected 293T cells, PSCA-transfected 293T cells, and LAPC-4 prostate cancer xenograft cells using an affinity purified polyclonal anti-PSCA antibody. Cells were not permeabilized in order to detect only surface expression. The y axis represents relative cell number and the x axis represents fluorescent staining intensity on a logarithmic scale.

FIG. 13. In situ hybridization of biotin-labeled PSCA probes to human metaphase cells from phytohemagglutinin-stimulated peripheral blood lymphocytes. The chromosome 8 homologues are identified with arrows; specific labeling was observed at 8q24.2. The inset shows partial karyotypes of two chromosome 8 homologues illustrating specific labeling at 8q24.2 (arrowheads). Images were obtained using a Zeiss Axiophot microscope coupled to a cooled charge coupled device (CCD) camera. Separate images of DAPI stained chromosomes and the hybridization signal were merged using image analysis software (NU200 and Image 1.57).

[**FIG. 14.** Flow Cytometric analysis of cell surface PSCA protein expression on prostate cancer xenograft (LAPC-9), prostate cancer cell line (LAPC-4) and normal prostate epithelial cells (PreC) using anti-PSCA monoclonal antibodies 1G8 (green) and 3E6 (red), mouse anti-PSCA polyclonal serum (blue), or control secondary antibody (black). See Example 5 for details.] **FIG. 14A.** Flow Cytometric analysis of cell surface PSCA expression on prostate cancer xenograft (LAPC-9) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

FIG. 14B. Flow Cytometric analysis of cell surface PSCA expression on prostate cancer cell line (LAPC-4) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

FIG 14C. Flow Cytometric analysis of cell surface PSCA expression on normal prostate epithelial cells (PreC) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

[**FIG. 15.** (a) An epitope map for each of the seven disclosed antibodies. (b) Epitope mapping of anti-PSCA monoclonal antibodies conducted by Western blot analysis of GST-PSCA fusion proteins.] **FIG. 15A.** An epitope map for each of the seven disclosed antibodies.

FIG 15B. Epitope mapping of anti-PSCA monoclonal antibodies conducted by Western blot analysis of GST-PSCA fusion proteins.

[FIG. 16. A schematic diagram showing that PSCA is a GPI-anchored protein.] FIG. 16A. Alignment of amino acid sequences of human PSCA, murine PSCA, and human stem cell antigen-2 (hSCA-2). Shaded regions highlight conserved amino acids.

FIG. 16B A schematic diagram showing that PSCA is a GPI-anchored protein.

FIG. 17. A photograph showing a FISH analysis of PSCA and c-myc Gene Copy No. in prostate cancer.

FIG. 18. A photograph showing FITC labeled 1G8 antibodies strongly bind PSCA protein on PSCA transfected LNCAP cells.

FIG. 19. A photograph showing FITC labeled 1G8 antibodies weakly bind PreC cells.

FIG. 20. A photograph showing in situ RNA hybridization of PSCA in normal prostate basal cells.

FIG. 21. PSCA immunostaining in primary prostate cancers. Representative paraffin-embedded sections from four patients were stained with anti-PSCA mAbs. The specimen from patient 1 demonstrates overexpression of PSCA protein in a Gleason grade 4 tumor (arrow) and undetectable expression of PSCA in adjacent normal glands (arrowhead) using PSCA mAb 1G8. The positively staining cancer completely surrounds the normal glands. The specimen from patient 2 demonstrates heterogeneous staining in a Gleason grade 3 + 3/4 cancer. The Gleason pattern 3 glands (arrowhead) stain weakly compared with the larger, more cribriform appearing Gleason pattern 3/4 glands (arrow). The specimen from patient 3 demonstrates strong expression of PSCA by a poorly differentiated Gleason 5 (arrow) tumor with mAb 1G8. Patient 4 is a biopsy specimen showing no PSCA staining in the majority of a poorly differentiated tumor (arrowhead)

and extremely weak staining in a cribriform focus identified in the specimen. The matched bone metastasis from patient 4 is shown in Figure 28.

FIG. 22. A photograph of a bone sample showing bone metastases of prostate cancer as determined by biotinylated 1G8 monoclonal antibody linked to horseradish peroxidase-conjugated streptavidin.

FIG. 23. A photograph of a bone sample showing bone metastases of prostate cancer as determined by biotinylated 1G8 monoclonal antibody linked to horseradish peroxidase-conjugated streptavidin.

FIG. 24. A photograph of a bone sample showing bone metastases of prostate cancer as determined by biotinylated 3E6 monoclonal antibody linked to horseradish peroxidase-conjugated streptavidin.

FIG. 25. A northern blot showing increased level of PSCA RNA in LAPC9 and transitional cell carcinoma of an advanced bladder carcinoma.

FIG. 26. A photograph of a tissue undergoing early stage prostate cancer as determined by biotinylated 3E6 monoclonal antibody linked to horseradish peroxidase-conjugated streptavidin.

FIG. 27. A photograph of a bone sample showing bone metastases of prostate cancer as determined by hematoxylin stained 3E6 monoclonal antibody.

FIG. 28. PSCA immunostaining in prostate cancer bone metastases. The top panel shows the hematoxylin and eosin (left) and PSCA (right) staining of a bony lesion from patient 5. A single focus suspicious for cancer (arrow) was identified in the H and E section and confirmed by intense staining with anti-PSCA mAb 1G8 (arrow). The bottom panel shows the H and E (left) and PSCA staining of a bone lesion from patient 4. The primary lesion from patient 4 is depicted in Figure 21. Both the H and E and PSCA stains

show diffuse bony involvement by prostate cancer (arrows). Again, PSCA immunostaining in the bone metastasis is uniform and intense.

FIG. 29. A photograph of a tissue undergoing early stage prostate cancer as determined by biotinylated 1G8 monoclonal antibody linked to horseradish peroxidase-conjugated streptavidin.

FIG. 30. A photograph showing that 1G8 binds LAPC9 cells as determined by hematoxylin staining.

FIG. 31. A photograph showing that 1G8 binds PSCA-transfected LnCaP cells.

FIG. 32. A photograph showing that 1G8 does not bind LnCaP cells (not transfected with PSCA).

FIG. 33. Flow cytometric recognition of PSCA on the cell surface of nonpermeabilized LAPC-9 human prostate cancer cells using mAbs 1G8, 2H9, 3E6, 3C5 and 4A10. Staining was compared to an irrelevant isotype control antibody.

FIG. 34. A photograph showing 293T cells transiently transfected with PSCA and immunoblotted with PSCA monoclonal antibodies. Monoclonal antibodies 2H9 and 3E6 binds deglycosylated PSCA but does not bind glycosylated PSCA in 293T cells. In contrast, monoclonal antibodies 1G8, 3C5,and 4A10 recognizes glycosylated PSCA.

FIG. 35. Immunofluorescent analysis demonstrating cell surface expression of PSCA in nonpermeabilized prostate cancer cells. LNCaP cells were stably transfected with PSCA and stained with mAbs 1G8, 3E6, 3C5 and 4A10. Negative controls included irrelevant isotype antibody and LNCaP cells transfected with control vector, all of which showed no staining even after prolonged exposures.

FIG. 36. A photograph showing monoclonal antibody 2H9 binds LAPC9 cells.

FIG. 37. A photograph showing immunological reactivity of anti-[PSCA] mAbs. [(A)] Immunoprecipitation of PSCA from 293T cells transiently transfected with PSCA using mAbs 1G8, 2H9, 3C5, 3E6 and 4A10. The control was an irrelevant murine IgG mAb. [(B) Immunoblot analysis of 293T cells transiently transfected with PSCA using the five anti-PSCA mAbs. mAbs 1G8, 3C5 and 4A10 all recognize equivalent molecular forms of PSCA, whereas mAbs 2H9 and 3E6 only weakly recognize deglycosylated forms of PSCA in 293T-PSCA cells in this assay.]

FIG. 38. Immunohistochemical staining of normal prostate with anti-PSCA mAbs. Examples shown include a normal gland stained with an irrelevant isotype antibody as a negative control (arrow), PSCA mAb 3E6 and mAb 1G8. PSCA mAb 3E6 preferentially stains basal cells (arrow) when compared with secretory cells (arrowhead), whereas mAb 1G8 stains both basal (arrow) and secretory (arrowhead) cells equally. Also shown is strong staining of an atrophic single-layered gland from a normal prostate specimen stained with PSCA mAb 2H9.

[**FIG. 39.** Expression of PSCA protein in normal tissues. (A) Panel *a* shows staining of bladder transitional epithelium with mAb 1G8. Panel *b* shows colonic neuroendocrine cell staining with mAb 1G8. Double staining with chromogranin confirmed that the positive cells are of neuroendocrine origin (not shown). Panel *c* shows staining of collecting ducts (arrow) and tubules with mAb 3E6. Panel *d* show staining of placental trophoblasts with mAb 3E6. (B) Northern blot analysis of PSCA mRNA expression. Total RNA from normal prostate, kidney, bladder and the LAPC-9 prostate cancer xenograft was analyzed using a PSCA specific probe (top panel). The same membrane was probed with actin to control of loading differences (bottom panel).] **FIG. 39A.** Expression of PSCA in normal tissues. Panel *a* shows staining of bladder transitional epithelium with mAb 1G8. Panel *b* shows colonic neuroendocrine cell staining with mAb 1G8. Double staining with chromogranin confirmed that the positive cells are of neuroendocrine origin (not shown). Panel *c* shows staining of collecting ducts (arrow)

and tubules with mAb 3E6. Panel *d* show staining of placental trophoblasts with mAb 3E6.

FIG. 39B. Expression of PSCA in normal tissues. Northern blot analysis of PSCA mRNA expression. Total RNA from normal prostate, kidney, bladder and the LAPC-9 prostate cancer xenograft was analyzed using a PSCA specific probe (top panel). The same membrane was probed with actin to control of loading differences (bottom panel).

[**FIG. 40.** Targeting of mouse PSCA gene. (A) Panel *a* is a schematic drawing showing a strategy for creating a PSCA targeting vector. (B) Panel b is a photograph of a southern blot analysis of genomic DNA using 3' probe showing recovery of wild-type (+/+) and heterozygous (+/-) ES cells.] **FIG. 40A.** Targeting of mouse PSCA gene. A schematic drawing showing a strategy for creating a PSCA targeting vector.

FIG. 40B. Targeting of mouse PSCA gene. A photograph of a Southern blot analysis of genomic DNA using 3' probe showing recovery of wild-type (+/+) and heterozygous (+/-) ES cells.

FIG. 41. The upper panel is a schematic drawing of a strategy for generating transgenic mouse models of prostate cancer. The lower panel is a list of existing transgenic mouse models of prostate cancer.

FIG. 42. A schematic drawing showing reporter gene constructs for transfection assay.

FIG. 43. A bar graph showing the tissue-predominant expression (prostate and bladder cells) of the 9kb human PSCA upstream regulatory region having increased gene expression activity.

FIG. 44. Bar graphs identifying prostate-predominant expression elements within PSCA upstream regions having increased gene expression activity, i.e., the 9kb, 6kb, 3kb, and 1kb PSCA regions.

FIG. 45. A schematic drawing showing the design of transgenic vectors containing either a 9 kb or 6 kb human PSCA upstream region operatively linked to a detectable marker.

FIG. 46. Photographs showing that the 9kb PSCA upstream region drives reporter gene expression in prostate, bladder and skin in vivo.

[**FIG. 47.** Photographs of multiple tissue northern blot analysis showing tissue specific expression patterns of human and murine PSCA RNA.] **FIG. 47A.** Photograph of a multiple tissue Northern blot analysis showing tissue specific expression patterns of human PSCA RNA.

FIG. 47B. Photograph of a multiple tissue Northern blot analysis showing tissue specific expression patterns of murine PSCA RNA.

[**FIG. 48.** Complete inhibition of LAPC-9 prostate tumor growth in SCID mice by treatment with anti-PSCA monoclonal antibodies. The upper panel represents mice injected with LAPC-9 s.c. and treated with a mouse IgG control, while in the lower panel mice were injected with LAPC-9 s.c. but treated with the anti-PSCA mAb cocktail. Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-A, infra. In the anti-PSCA group, an arbitrary value of 20 was given for all data points to create a line, although the actual tumor volume was 0 (Example 18-A, infra).] **FIG. 48A.** Complete inhibition of LAPC-9 prostate tumor growth in SCID mice by treatment with anti-PSCA monoclonal antibodies. Mice were injected with LAPC-9 s.c. and treated with a mouse IgG control. Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-A, infra.

FIG. 48B. Complete inhibition of LAPC-9 prostate tumor growth in SCID mice by treatment with anti-PSCA monoclonal antibodies. Mice were injected with LAPC-9 s.c.

and treated with the anti-PSCA mAb cocktail. Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-A, infra. In the anti-PSCA group, an arbitrary value of 20 was given for all data points to create a line, although the actual tumor volume was 0 (Example 18-A, infra).

[**FIG. 49.** Characteristics of anti-PSCA monoclonal antibodies utilized in the in vivo tumor challenge study described in Example 18. (A) Isotype and epitope map: The region of PSCA protein recognized by the anti-PSCA mAbs was determined by ELISA analysis using GST-fusion proteins (50ng/well) encoding the indicated amino acids of PSCA. Following incubation of wells with hybridoma supernatants, anti-mouse-HRP conjugate antibody was added and reactivity was determined by the addition of 3,3' 5,5'-Tetramethylbenzidine base (TMB) substrate. Optical densities (450nm) are the means of duplicate determinations. (B) Epitope map determined by Western analysis: 50ng of the indicated GST-PSCA fusion protein was separated by SDS-PAGE and transferred to nitrocellulose. Western analysis was carried out by incubation of blots with hybridoma supernatants followed by anti-mouse-HRP secondary Ab and visualized by enhanced chemiluminesence.] **FIG. 49A.** Characteristics of anti-PSCA monoclonal antibodies utilized in the in vivo tumor challenge study described in Example 18. Isotype and epitope map: The region of PSCA protein recognized by the anti-PSCA mAbs was determined by ELISA analysis using GST-fusion proteins (50ng/well) encoding the indicated amino acids of PSCA. Following incubation of wells with hybridoma supernatants, anti-mouse-HRP conjugate antibody was added and reactivity was determined by the addition of 3,3' 5,5'- Tetramethylbenzidine base (TMB) substrate. Optical densities (450nm) are the means of duplicate determinations.

FIG. 49B. Characteristics of anti-PSCA monoclonal antibodies utilized in the in vivo tumor challenge study described in Example 18. Epitope map determined by Western analysis: 50ng of the indicated GST-PSCA fusion protein was separated by SDS-PAGE and transferred to nitrocellulose. Western analysis was carried out by incubation of blots with hybridoma supernatants followed by anti-mouse-HRP secondary Ab and visualized by enhanced chemiluminesence.

[**FIG. 50.** Schematic representations of PSCA Capture ELISA. (A) Standardization and control antigens: A GST-fusion protein encoding amino acids 18-98 of the PSCA protein is used for generating a standard curve for quantification of unknown samples. Also depicted are approximate epitope binding regions of the anti-PSCA monoclonal and polyclonal antibodies used in the ELISA. A secreted recombinant mammalian expressed form of PSCA is used for quality control of the ELISA assay. This protein contains an Ig leader sequence to direct secretion of the recombinant protein and MYC and 6XHIS epitope tags for affinity purification. (B) ELISA format schematic.] **FIG. 50A.** Schematic representations of PSCA Capture ELISA. Standardization and control antigens: A GST-fusion protein encoding amino acids 18-98 of the PSCA protein is used for generating a standard curve for quantification of unknown samples. Also depicted are approximate epitope binding regions of the anti-PSCA monoclonal and polyclonal antibodies used in the ELISA. A secreted recombinant mammalian expressed form of PSCA is used for quality control of the ELISA assay. This protein contains an Ig leader sequence to direct secretion of the recombinant protein and MYC and 6XHIS epitope tags for affinity purification.

FIG. 50B. Schematic representations of PSCA Capture ELISA. An ELISA format schematic.

[**FIG. 51.** Quantification of recombinant secreted PSCA protein. (A) PSCA capture ELISA standard curve. (B) Quantification of PSCA protein secreted by mammalian cells. 2 day conditioned tissue culture supernatants from either 293T cells transfected with empty vector or with vector encoding recombinant secreted PSCA (secPSCA) was mixed with an equal volume of either PBS or normal human serum (Omega Scientific) and analyzed for the presence of PSCA protein. Data are the means of duplicate determinations ± range. ND not detectable.] **FIG. 51A.** Quantification of recombinant secreted PSCA protein. PSCA capture ELISA standard curve.

FIG. 51B. Quantification of PSCA protein secreted by mammalian cells. 2 day conditioned tissue culture supernatants from either 293T cells transfected with empty vector or with vector encoding recombinant secreted PSCA (secPSCA) was mixed with an equal volume of either PBS or normal human serum (Omega Scientific) and analyzed for the presence of PSCA protein. Data are the means of duplicate determinations + range. ND not detectable.

FIG. 52. Immunohistochemical Analysis of cell pellet, LAPC9AD xenograft, a BPH sample, and a prostate carcinoma tissue using anti-PSCA monoclonal antibody 3C5.

[FIG. 53. Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies. The top panel represents mice injected with 1 x 10^6 LAPC-9 s.c. and treated with a mouse IgG control (n = 10), the middle panel represents mice injected with LAPC-9 s.c. and treated with anti-PSCA mAb cocktail (n = 10), the bottom panel represents mice injected with LAPC-9 s.c. and treated with bovine IgG (n = 5). Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-B.]

FIG. 53A. Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies. Mice were injected with 1 x 10^6 LAPC-9 s.c. and treated with a mouse IgG control (n = 10). Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-B.

FIG. 53B. Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies. Mice were injected with LAPC-9 s.c. and treated with anti-PSCA mAb cocktail (n = 10). Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-B.

FIG. 53C. Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies. Mice were injected with LAPC-9 s.c. and treated with bovine IgG (n = 5). Each data point represents the ellipsoidal volume of tumors at specified time points as described in Example 18-B.

[**FIG. 54.** Inhibition of LAPC-9 tumor growth by the anti-PSCA monoclonal antibody 1G8. The upper panel represents mice injected with 1 x 10^6 LAPC-9 s.c. and treated with a mouse IgG control (n = 6), while in the lower panel mice were injected with LAPC-9 s.c. but treated with the anti-PSCA mAb 1G8 (n = 7). Each data point represents the ellipsoidal volume of tumors at specified time points.] **FIG. 54A.** Inhibition of LAPC-9 tumor growth by the anti-PSCA monoclonal antibody 1G8. Mice were injected with 1 x 10^6 LAPC-9 s.c. and treated with a mouse IgG control (n = 6). Each data point represents the ellipsoidal volume of tumors at specified time points.

FIG. 54B. Inhibition of LAPC-9 tumor growth by the anti-PSCA monoclonal antibody 1G8. Mice were injected with LAPC-9 s.c. and treated with the anti-PSCA mAb 1G8 (n = 7). Each data point represents the ellipsoidal volume of tumors at specified time points.

[**FIG. 55.** Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies 2A2 and 2H9. The upper panel represents mice injected with 1 x 10^6 LAPC-9 s.c. and treated with either a mouse IgG control (n = 6) or the 2A2 mAb (n = 7). The lower panel represents mice injected with LAPC-9 s.c. and treated with the same mouse IgG control (n = 6) or the 2H9 mAb (n = 7). All data points represent the mean ellipsoidal volume of tumors (mm^3) at the specified time points. Error bars represent standard error of the mean (SEM).] **FIG. 55A.** Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies 2A2 and 2H9. Mice were injected with 1 x 10^6 LAPC-9 s.c. and treated with either a mouse IgG control (n = 6) or the 2A2 mAb (n = 7). All data points represent the mean ellipsoidal volume of tumors (mm^3) at the specified time points. Error bars represent standard error of the mean (SEM).

FIG. 55B. Inhibition of LAPC-9 tumor growth by anti-PSCA monoclonal antibodies 2A2 and 2H9. Mice were injected with LAPC-9 s.c. and treated with the same mouse IgG control (n = 6) or the 2H9 mAb (n = 7). All data points represent the mean ellipsoidal volume of tumors (mm^3) at the specified time points. Error bars represent standard error of the mean (SEM).

[**FIG. 56.** Circulating PSA levels in LAPC-9 tumor-injected mice after treatment with anti-PSCA mAbs 2A2 and 2H9. The upper panel represents the mice injected with 1 x 10^6 LAPC-9 s.c. and treated with either the mouse IgG control (n = 6) or the 2A2 mAb (n = 7). The lower panel represents mice injected with LAPC-9 s.c. but treated with either the same mouse IgG control (n = 6) or the 2H9 mAb (n = 7). Each data point represents the mean PSA level determined from the serum of mice at weekly time points. Error bars represent standard error of the mean (SEM).] **FIG. 56A.** Circulating PSA levels in LAPC-9 tumor-injected mice after treatment with anti-PSCA mAbs 2A2 and 2H9. Mice were injected with 1 x 10^6 LAPC-9 s.c. and treated with either the mouse IgG control (n = 6) or the 2A2 mAb (n = 7). Each data point represents the mean PSA level determined from the serum of mice at weekly time points. Error bars represent standard error of the mean (SEM).

FIG. 56B. Circulating PSA levels in LAPC-9 tumor-injected mice after treatment with anti-PSCA mAbs 2A2 and 2H9. Mice were injected with LAPC-9 s.c. and treated with either the same mouse IgG control (n = 6) or the 2H9 mAb (n = 7). Each data point represents the mean PSA level determined from the serum of mice at weekly time points. Error bars represent standard error of the mean (SEM).

FIG. 57. Inhibition of established LAPC-9 prostate cancer xenografts by PSCA monoclonal antibody 3C5. See Example 18-C4 for details.

[**FIG. 58.** Amino acid sequence of the heavy chain variable domain regions of PSCA monoclonal antibodies 1G8. CDRs are labeled and underlined.] **FIG. 58.** Nucleotide sequence (SEQ ID NO:10) and amino acid sequence (SEQ ID NO:11) of the heavy chain variable domain regions of PSCA monoclonal antibodies 1G8. CDRs are labeled and underlined.

[**FIG. 59.** Amino acid sequence of the heavy chain variable domain regions of PSCA monoclonal antibodies 4A10. CDRs are labeled and underlined.] **FIG. 59.** Nucleotide sequence (SEQ ID NO:12) and amino acid sequence (SEQ ID NO:13) of the heavy chain

variable domain regions of PSCA monoclonal antibodies 4A10. CDRs are labeled and underlined.

[FIG. 60. Amino acid sequence of the heavy chain variable domain regions of PSCA monoclonal antibodies 2H9. CDRs are labeled and underlined.] FIG. 60. Nucleotide sequence (SEQ ID NO:14) and amino acid sequence (SEQ ID NO:15) of the heavy chain variable domain regions of PSCA monoclonal antibodies 2H9. CDRs are labeled and underlined.

FIG. 61. Amino acid sequence alignments of CDRs of PSCA mAbs 1G8, 4A10 and 2H9.

[FIG. 62. Photographs showing PSCA protein expression in normal bladder and various bladder carcinoma tissues using immunohistochemical staining of paraffin-embedded samples with PSCA mAb 1G8.] FIG. 62A. Photograph showing PSCA protein expression in normal bladder tissue using immunohistochemical staining of paraffin-embedded samples with PSCA mAb 1G8.

FIG. 62B. Photograph showing PSCA protein expression in non-invasive superficial papillar tissue using immunohistochemical staining of paraffin-embedded samples with PSCA mAb 1G8.

FIG. 62C. Photograph showing PSCA protein expression in carcinoma in situ, a high grade pre-cancerous lesion, using immunohistochemical staining of paraffin-embedded samples with PSCA mAb 1G8.

FIG. 62D. Photograph showing PSCA protein expression in invasive bladder cancer tissue using immunohistochemical staining of paraffin-embedded samples with PSCA mAb 1G8.

FIG. 63. Northern blot analysis of PSCA expression in several pancreatic cancer cells lines. Northern blot analysis of PSCA expression in normal prostate and several prostate cancer xenografts are shown alongside for comparison. RNA levels between all samples were normalized.

FIG. 64. Western blot analysis of PSCA protein expression in prostate and pancreatic cancer cell line using PSCA mab 1G8.

[**FIG. 65.** PSCA mAbs exert growth inhibitory effect through PSCA protein. The growth inhibitory effect of PSCA mAb 1G8 on LAPC-9 and PC-3 prostate tumors is compared, showing no effect on PC-3 tumors, which do not express PSCA antigen, but significant growth inhibition in LAPC-9 tumors, which do express PSCA antigen. See Examples 18-C1, -C3 for details.] **FIG. 65A.** PSCA mAbs exert growth inhibitory effect through PSCA protein. The growth inhibitory effect of PSCA mAb 1G8 on LAPC-9 prostate tumors showing significant growth inhibition in LAPC-9 tumors, which express PSCA antigen. See Examples 18-C1, -C3 for details.

FIG. 65B. PSCA mAbs exert growth inhibitory effect through PSCA protein. The growth inhibitory effect of PSCA mAb 1G8 on PC-3 prostate tumors, showing no effect on PC-3 tumors, which do not express PSCA antigen. See Examples 18-C1, -C3 for details.

[**FIG. 66.** Growth inhibition of established LAPC-9 (AD) orthotopic tumors by the anti-PSCA mAb 1G8. (A) Mice having low levels of serum PSA. Two mg of 1G8 was administered to these mice on days 10, 13, and 15, followed by one mg on days 17, 20, 22, 25, 27, 29, 34, 41, and 49 as indicated by the arrows. (B) Mice having moderate levels of serum PSA. One mg of 1G8 was administered on days 12, 13, 14, 19, 20, 22, 25, 27, 29, and 33 as indicated by the arrows.] **FIG. 66A.** Growth inhibition of established LAPC-9 (AD) orthotopic tumors by the anti-PSCA mAb 1G8. Mice having low levels of serum PSA. Two mg of 1G8 was administered to these mice on days 10,

13, and 15, followed by one mg on days 17, 20, 22, 25, 27, 29, 34, 41, and 49 as indicated by the arrows.

FIG. 66B. Growth inhibition of established LAPC-9 (AD) orthotopic tumors by the anti-PSCA mAb 1G8. Mice having moderate levels of serum PSA. One mg of 1G8 was administered on days 12, 13, 14, 19, 20, 22, 25, 27, 29, and 33 as indicated by the arrows.

[**FIG. 67.** Treatment with the anti-PSCA mAb, 1G8, increases survival of mice bearing established LAPC-9 (AD) orthotopic tumors. (A) The mice in Figure 66 A, which were treated with 1G8, exhibited an increase in survival compared to mice treated with PBS. (B) The mice in Figure 66 B, which were treated with 1G8, exhibited an increase in survival compared to mice treated with PBS.] **FIG. 67A.** Treatment with the anti-PSCA mAb, 1G8, increases survival of mice bearing established LAPC-9 (AD) orthotopic tumors. Mice treated with 1G8 exhibited an increase in survival compared to mice treated with PBS.

FIG. 67B. Treatment with the anti-PSCA mAb, 1G8, increases survival of mice bearing established LAPC-9 (AD) orthotopic tumors. Mice treated with PBS.

[**FIG. 68.** Growth inhibition of established LAPC-9 AD orthotopic tumors by the anti-PSCA mAb 3C5. (A) One mg of 3C5 was administered to tumor-bearing mice on days 6, 8, 10, 13, 15, 17, 20, 22, 24, and 29 as indicated by the arrows. The mice were bled on the days indicated on the X-axis for PSA determinations. (B) Two mg of 3C5 was administered to tumor-bearing mice on days 9, 12, and 15, followed by one mg on days 18, 20, 22, 25, 27, and 29 as indicated by the arrows. The mice were bled on the days indicated on the X-axis for PSA determinations.] **FIG. 68A.** Growth inhibition of established LAPC-9 AD orthotopic tumors by the anti-PSCA mAb 3C5. One mg of 3C5 was administered to tumor-bearing mice on days 6, 8, 10, 13, 15, 17, 20, 22, 24, and 29 as indicated by the arrows. The mice were bled on the days indicated on the X-axis for PSA determinations.

FIG. 68B. Growth inhibition of established LAPC-9 AD orthotopic tumors by the anti-PSCA mAb 3C5. Two mg of 3C5 was administered to tumor-bearing mice on days 9, 12, and 15, followed by one mg on days 18, 20, 22, 25, 27, and 29 as indicated by the arrows. The mice were bled on the days indicated on the X-axis for PSA determinations.

[**FIG. 69.** Treatment with the anti-PSCA mAb, 3C5, increases survival of mice bearing LAPC-9 AD orthotopic tumors. (A) The mice in Figure 68 A, which were treated with 3C5, exhibited an increase in survival compared to mice treated with PBS. There were 4 mice in the PBS-treated group and 5 mice in the 3C5-treated group. (B) The mice in Figure 68 B, which were treated with 3C5, exhibited an increase in survival compared to mice treated with PBS. There were 6 mice in both the PBS-treated and 3C5-treated groups.] **FIG. 69A.** Treatment with the anti-PSCA mAb, 3C5, increases survival of mice bearing LAPC-9 AD orthotopic tumors. Mice treated with 3C5 exhibited an increase in survival compared to mice treated with PBS. There were 4 mice in the PBS-treated group and 5 mice in the 3C5-treated group.

FIG. 69B. Treatment with the anti-PSCA mAb, 3C5, increases survival of mice bearing LAPC-9 AD orthotopic tumors. Mice treated with 3C5 exhibited an increase in survival compared to mice treated with PBS. There were 6 mice in both the PBS-treated and then 3C5-treated groups.

FIG. 70. Growth inhibition of established PC3-PSCA tumors by 1G8 alone or in combination with doxorubicin. One mg of 1G8 was administered to tumor-bearing mice on days 9, 11, 14, 16, 18, 21, 23, 25, and 28 as indicated by the arrows. Twenty-five micrograms of doxorubicin was administered on days 9, 16, and 23 as indicated by the (•) symbol.

[**FIG. 71.** Anti-PSCA antibody administered to tumor-bearing mice circulates and targets tumors expressing PSCA A) Immunohistochemistry of a tumor explant from a mouse, bearing an established PSCA-expressing tumor, treated with 3C5. B) Immunohistochemistry of a tumor explant from a mouse, bearing an established PSCA-

expressing tumor, treated with mouse IgG.] **FIG. 71A.** Anti-PSCA antibody administered to tumor-bearing mice circulates and targets tumors expressing PSCA. Immunohistochemistry of a tumor explant from a mouse, bearing an established PSCA-expressing tumor, treated with 3C5.

FIG. 71B. Anti-PSCA antibody administered to tumor-bearing mice circulates and targets tumors expressing PSCA. Immunohistochemistry of a tumor explant from a mouse, bearing an established PSCA-expressing tumor, treated with mouse IgG.

FIG. 72. Anti-PSCA antibody administered to a tumor-bearing mouse circulates and targets tumors expressing PSCA. A Western blot analysis of tumor lysates from tumors explanted from mice described in Figure 71, probed with goat anti-mouse IgG-HRP antibody.

FIG. 73. Anti-PSCA antibody administered to a tumor-bearing mouse circulates and targets tumors expressing PSCA. A Western blot analysis of tumor lysates from tumors explanted from mice bearing established PSCA-expressing tumors, treated with 1G8. The blot was probed with goat anti-mouse IgG-HRP antibody.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to Prostate Stem Cell Antigen (hereinafter "PSCA"). PSCA is a novel, glycosylphosphatidylinositol (GPI)-anchored cell surface antigen which is expressed in normal cells such prostate cells, urothelium, renal collecting ducts, colonic neuroendocrine cells, placenta, normal bladder and urethral transitional epithelial cells (FIG. 16). PSCA, in addition to normal cells, is also overexpressed by both androgen-dependent and androgen-independent prostate cancer cells (FIG. 9-11), prostate cancer metastases to bone (FIG. 20-24 and 26-32), bladder carcinomas (FIG. 6, 25 and 62), and pancreatic carcinomas (FIGS. 63 and 64). The expression of PSCA in cancer, e.g., prostate cancer and bladder cancer, appears to correlate with increasing grade. Further, overexpression

of PSCA (i.e. higher expression than found in normal cells) in patients suffering from cancer, e.g., prostate cancer, appears to be indicative of poor prognosis.

PSCA mRNA is also expressed by a subset of basal cells in normal prostate. The basal cell epithelium is believed to contain the progenitor cells for the terminally differentiated secretory cells (Bonkhoff et al., 1994, Prostate 24: 114-118). Recent studies using cytokeratin markers suggest that the basal cell epithelium contains at least two distinct cellular subpopulations, one expressing cytokeratins 5 and 14 and the other cytokeratins 5, 8 and 18 (Bonkhoff and Remberger, 1996, Prostate 28: 98-106). The finding that PSCA is expressed by only a subset of basal cells suggests that PSCA may be a marker for one of these two basal cell lineages.

The biological function of PSCA is unknown. The Ly-6 gene family is involved in diverse cellular functions, including signal transduction and cell-cell adhesion. Signaling through SCA-2 has been demonstrated to prevent apoptosis in immature thymocytes (Noda et al., 1996, J. Exp. Med. 183: 2355-2360). Thy-1 is involved in T cell activation and transmits signals through src-like tyrosine kinases (Thomas et al., 1992, J. Biol. Chem. 267: 12317-12322). Ly-6 genes have been implicated both in tumorigenesis and in homotypic cell adhesion (Bamezai and Rock, 1995, Proc. Natl. Acad. Sci. USA 92: 4294-4298; Katz et al., 1994, Int. J. Cancer 59: 684-691; Brakenhoff et al., 1995, J. Cell Biol. 129: 1677-1689). Based on its restricted expression in basal cells and its homology to Sca-2, we hypothesize that PSCA may play a role in stem/progenitor cell functions such as self-renewal (anti-apoptosis) and/or proliferation.

PSCA is highly conserved in mice and humans. The identification of a conserved gene which is predominantly restricted to prostate supports the hypothesis that PSCA may play an important role in normal prostate development.

In its various aspects, as described in detail below, the present invention provides PSCA proteins, antibodies, nucleic acid molecules, recombinant DNA molecules, transformed host

cells, generation methods, assays, immunotherapeutic methods, transgenic animals, immunological and nucleic acid-based assays, and compositions.

PSCA PROTEINS

One aspect of the invention provides various PSCA proteins and peptide fragments thereof. As used herein, PSCA refers to a protein that has the amino acid sequence of human PSCA (SEQ ID NO:2) as provided in FIGS. 1B and 3, the amino acid sequence of the murine PSCA homologue (SEQ ID NO:4) as provided in FIG. 3, or the amino acid sequence of other mammalian PSCA homologues, as well as allelic variants and conservative substitution mutants of these proteins that have PSCA activity. The PSCA proteins of the invention include the specifically identified and characterized variants herein described, as well as allelic variants, conservative substitution variants and homologs that can be isolated/generated and characterized without undue experimentation following the methods outlined below. For the sake of convenience, all PSCA proteins will be collectively referred to as the PSCA proteins, the proteins of the invention, or PSCA.

The term "PSCA" includes all naturally occurring allelic variants, isoforms, and precursors of human PSCA (SEQ ID NO:2) as provided in FIGS. 1B and 3 and murine PSCA (SEQ ID NO:4) as provided in FIG. 3. In general, for example, naturally occurring allelic variants of human PSCA will share significant homology (e.g., 70 - 90%) to the PSCA amino acid sequence provided in FIGS. 1B and 3. Allelic variants, though possessing a slightly different amino acid sequence, may be expressed on the surface of prostate cells as a GPI linked protein or may be secreted or shed. Typically, allelic variants of the PSCA protein will contain conservative amino acid substitutions from the PSCA sequence herein described or will contain a substitution of an amino acid from a corresponding position in a PSCA homologue such as, for example, the murine PSCA homologue described herein.

One class of PSCA allelic variants will be proteins that share a high degree of homology with at least a small region of the PSCA amino acid sequences presented in FIGS. 1B (SEQ ID NO:2) and 3 (SEQ ID NO: 2 or 4), but will further contain a radical departure from the

sequence, such as a non-conservative substitution, truncation, insertion or frame shift. Such alleles are termed mutant alleles of PSCA and represent proteins that typically do not perform the same biological functions.

Conservative amino acid substitutions can frequently be made in a protein without altering either the conformation or the function of the protein. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

The amino acid sequence of human PSCA protein (SEQ ID NO:2) is provided in FIGS. 1B and 3. Human PSCA is comprised of a single subunit of 123 amino acids and contains an amino-terminal signal sequence, a carboxy-terminal GPI-anchoring sequence, and multiple N-glycosylation sites. PSCA shows 30% homology to stem cell antigen-2 (SCA-2), a member of the Thy-1/Ly-6 gene family, a group of cell surface proteins which mark the earliest phases of hematopoetic development. The amino acid sequence of a murine PSCA homologue (SEQ ID NO:4) is shown in FIG. 3. Murine PSCA is a single subunit protein of 123 amino acids having approximately 70% homology to human PSCA and similar structural organization.

PSCA proteins may be embodied in many forms, preferably in an isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are

employed to remove the PSCA protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated PSCA protein. A purified PSCA protein molecule will be substantially free of other proteins or molecules that impair the binding of PSCA to antibody or other ligand. The nature and degree of isolation and purification will depend on the intended use. Embodiments of the PSCA protein include a purified PSCA protein and a functional, soluble PSCA protein. One example of a functional soluble PSCA protein has the amino acid sequence shown in FIG. 1B (SEQ ID NO:2) or a fragment thereof. In one form, such functional, soluble PSCA proteins or fragments thereof retain the ability to bind antibody or other ligand.

The invention also provides peptides comprising biologically active fragments of the human (SEQ ID NO:2) and murine (SEQ ID NO:4) PSCA amino acid sequences shown in FIGS. 1B and 3. For example, the invention provides a peptide fragment having the amino acid sequence TARIRAVGLLTVISK (SEQ ID NO:16), a peptide fragment having the amino acid sequence VDDSQDYYVGKK (SEQ ID NO:17), and SLNCVDDSQDYYVGK (SEQ ID NO:18).

The peptides of the invention exhibit properties of PSCA, such as the ability to elicit the generation of antibodies that specifically bind an epitope associated with PSCA. Such peptide fragments of the PSCA proteins can be generated using standard peptide synthesis technology and the amino acid sequences of the human or murine PSCA proteins disclosed herein. Alternatively, recombinant methods can be used to generate nucleic acid molecules that encode a fragment of the PSCA protein. In this regard, the PSCA-encoding nucleic acid molecules described herein provide means for generating defined fragments of PSCA.

As discussed below, peptide fragments of PSCA are particularly useful in: generating domain specific antibodies; identifying agents that bind to PSCA or a PSCA domain; identifying cellular factors that bind to PSCA or a PSCA domain; and isolating homologs or other allelic forms of human PSCA. PSCA peptides containing particularly interesting structures can be predicted and/or identified using various analytical techniques well known

in the art, including, for example, the methods of Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis, or on the basis of immunogenicity. As examples, hydrophobicity and Chou-Fasman plots of human PSCA are provided in FIGS. 4 and 5, respectively. Fragments containing such residues are particularly useful in generating subunit specific anti-PSCA antibodies or in identifying cellular factors that bind to PSCA.

Various regions of the PSCA protein can bind to anti-PSCA antibodies. The regions of the PSCA protein may include, for example, the N-terminal region, middle region, and C-terminal region (Example 18, Figure 49). The N-terminal region includes any portion of the PSCA protein encompassed by amino acid residues 2-50, preferably residues 18-50. The middle region includes any portion of the PSCA protein encompassed by amino acid residues 46-109, preferably residues 46-98. The C-terminal region includes any portion of the PSCA protein encompassed by amino acid residues 85-123, preferably residues 85-98.

The PSCA proteins of the invention may be useful for a variety of purposes, including but not limited to their use as diagnostic and/or prognostic markers of prostate cancer, the ability to elicit the generation of antibodies, and as targets for various therapeutic modalities, as further described below. PSCA proteins may also be used to identify and isolate ligands and other agents that bind to PSCA. In the normal prostate, PSCA is expressed exclusively in a subset of basal cells, suggesting that PSCA may be used as a marker for a specific cell lineage within basal epithelium. In addition, the results herein suggest that this set of basal cells represent the target of neoplastic transformation. Accordingly for example, therapeutic strategies designed to eliminate or modulate the molecular factors responsible for transformation may be specifically directed to this population of cells via the PSCA cell surface protein.

PSCA ANTIBODIES

The invention further provides antibodies (e.g., polyclonal, monoclonal, chimeric, and humanized antibodies) that bind to PSCA. The most preferred antibodies will selectively

bind to PSCA and will not bind (or will bind weakly) to non-PSCA proteins. The most preferred antibodies will specifically bind to PSCA. It is intended that the term "specifically bind" means that the antibody predominantly binds to PSCA. Anti-PSCA antibodies that are particularly contemplated include monoclonal and polyclonal antibodies as well as fragments thereof (e.g., recombinant proteins) containing the antigen binding domain and/or one or more complement determining regions of these antibodies. These antibodies can be from any source, e.g., rat, dog, cat, pig, horse, mouse or human.

In one embodiment, the PSCA antibodies specifically bind to the extracellular domain of a PSCA protein, e.g., on the cell surface of prostate cancer cells from primary lesions and prostate cancer bone metastases. It is intended that the term "extracellular domain" means any portion of the PSCA protein which is exterior to the plasma membrane of the cell. In other embodiments, the PSCA antibodies specifically bind to other domains of a PSCA protein or precursor (such as a portion of the N-terminal region, the middle region, or the C-terminal region; Figure 49). As will be understood by those skilled in the art, the regions or epitopes of a PSCA protein to which an antibody is directed may vary with the intended application. For example, antibodies intended for use in an immunoassay for the detection of membrane-bound PSCA on viable prostate cancer cells should be directed to an accessible epitope on membrane-bound PSCA. Examples of such antibodies are described the Examples which follow. Antibodies that recognize other epitopes may be useful for the identification of PSCA within damaged or dying cells, for the detection of secreted PSCA proteins or fragments thereof. The invention also encompasses antibody fragments that specifically recognize a PSCA protein. As used herein, an antibody fragment is defined as at least a portion of the variable region of the immunoglobulin molecule that binds to its target, i.e., the antigen binding region. Some of the constant region of the immunoglobulin may be included.

For example, the overexpression of PSCA in both androgen-dependent and androgen-independent prostate cancer cells, and the cell surface location of PSCA represent characteristics of an excellent marker for screening, diagnosis, prognosis, and follow-up assays and imaging methods. In addition, these characteristics indicate that PSCA may

be an excellent target for therapeutic methods such as targeted antibody therapy, immunotherapy, and gene therapy.

PSCA antibodies of the invention may be particularly useful in diagnostic assays, imaging methodologies, and therapeutic methods in the management of prostate cancer. The invention provides various immunological assays useful for the detection of PSCA proteins and for the diagnosis of prostate cancer. Such assays generally comprise one or more PSCA antibodies capable of recognizing and binding a PSCA protein, and include various immunological assay formats well known in the art, including but not limited to various types of precipitation, agglutination, complement fixation, radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA) (H. Liu et al. Cancer Research 58: 4055-4060 (1998), immunohistochemical analysis and the like. In addition, immunological imaging methods capable of detecting prostate cancer are also provided by the invention, including but limited to radioscintigraphic imaging methods using labeled PSCA antibodies. Such assays may be clinically useful in the detection, monitoring, and prognosis of prostate cancer.

In one embodiment, PSCA antibodies and fragments thereof (e.g., Fv, Fab', F(ab')2) are used for detecting the presence of a prostate cancer, bladder carcinoma, pancreatic carcinoma, bone metastases of prostate cancer, PIN, or basal epithelial cell expressing a PSCA protein. The presence of such PSCA positive (+) cells within various biological samples, including serum, prostate and other tissue biopsy specimens, other tissues such as bone, urine, etc., may be detected with PSCA antibodies. In addition, PSCA antibodies may be used in various imaging methodologies, such as immunoscintigraphy with Induim-111 (or other isotope) conjugated antibody. For example, an imaging protocol similar to the one recently described using an In-111 conjugated anti-PSMA antibody may be used to detect recurrent and metastatic prostate carcinomas (Sodee et al., 1997, Clin Nuc Med 21: 759-766). In relation to other markers of prostate cancer, such as PSMA for example, PSCA may be particularly useful for targeting androgen independent prostate cancer cells. PSCA antibodies may also be used therapeutically to inhibit PSCA function.

PSCA antibodies may also be used in methods for purifying PSCA proteins and peptides and for isolating PSCA homologues and related molecules. For example, in one embodiment, the method of purifying a PSCA protein comprises incubating a PSCA antibody, which has been coupled to a solid matrix, with a lysate or other solution containing PSCA under conditions which permit the PSCA antibody to bind to PSCA; washing the solid matrix to eliminate impurities; and eluting the PSCA from the coupled antibody. Additionally, PSCA antibodies may be used to isolate PSCA positive cells using cell sorting and purification techniques. The presence of PSCA on prostate tumor cells (alone or in combination with other cell surface markers) may be used to distinguish and isolate human prostate cancer cells from other cells. In particular, PSCA antibodies may be used to isolate prostate cancer cells from xenograft tumor tissue, from cells in culture, etc., using antibody-based cell sorting or affinity purification techniques. Other uses of the PSCA antibodies of the invention include generating anti-idiotypic antibodies that mimic the PSCA protein, e.g., a monoclonal anti-idiotypic antibody reactive with an idiotype on any of the monoclonal antibodies of the invention such as 1G8, 2A2, 2H9, 3C5, 3E6, 3G3, and 4A10.

The ability to generate large quantities of relatively pure human PSCA positive prostate cancer cells which can be grown in tissue culture or as xenograft tumors in animal models (e.g., SCID or other immune deficient mice) provides many advantages, including, for example, permitting the evaluation of various transgenes or candidate therapeutic compounds on the growth or other phenotypic characteristics of a relatively homogeneous population of prostate cancer cells. Additionally, this feature of the invention also permits the isolation of highly enriched preparations of human PSCA positive prostate cancer specific nucleic acids in quantities sufficient for various molecular manipulations. For example, large quantities of such nucleic acid preparations will assist in the identification of rare genes with biological relevance to prostate cancer disease progression.

Another valuable application of this aspect of the invention is the ability to isolate, analyze and experiment with relatively pure preparations of viable PSCA positive prostate tumor cells cloned from individual patients with locally advanced or metastatic disease. In this way, for example, an individual patient's prostate cancer cells may be expanded from a limited biopsy sample and then tested for the presence of diagnostic and prognostic genes, proteins, chromosomal aberrations, gene expression profiles, or other relevant genotypic and phenotypic characteristics, without the potentially confounding variable of contaminating cells. In addition, such cells may be evaluated for neoplastic aggressiveness and metastatic potential in animal models. Similarly, patient-specific prostate cancer vaccines and cellular immunotherapeutics may be created from such cell preparations.

Various methods for the preparation of antibodies are well known in the art. For example, antibodies may be prepared by immunizing a suitable mammalian host using a PSCA protein, peptide, or fragment, in isolated or immunoconjugated form (Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). In addition, fusion proteins of PSCA may also be used, such as a PSCA GST-fusion protein. Cells expressing or overexpressing PSCA may also be used for immunizations. Similarly, any cell engineered to express PSCA may be

used. This strategy may result in the production of monoclonal antibodies with enhanced capacities for recognizing endogenous PSCA. For example, using standard technologies described in Example 5 and standard hybridoma protocols (Harlow and Lane, 1988, Antibodies: A Laboratory Manual. (Cold Spring Harbor Press)), hybridomas producing monoclonal antibodies designated 1G8 (ATCC No. HB-12612), 2A2 (ATCC No.HB-12613), 2H9 (ATCC No.HB-12614), 3C5 (ATCC No. HB-12616), 3E6 (ATCC No.HB-12618), and 3G3 (ATCC No. HB-12615), 4A10 (ATCC No. HB-12617) were generated. These antibody were deposited on December 11, 1998 with the American Type Culture Collection (ATCC), [12301 Parklawn Drive, Rockville, MD 20852] 10801 University Boulevard, Manassas, VA 20110-2209.

Chimeric antibodies of the invention are immunoglobulin molecules that comprise a human and non-human portion. The antigen combining region (variable region) of a chimeric antibody can be derived from a non-human source (e.g. murine) and the constant region of the chimeric antibody which confers biological effector function to the immunoglobulin can be derived from a human source. The chimeric antibody should have the antigen binding specificity of the non-human antibody molecule and the effector function conferred by the human antibody molecule.

In general, the procedures used to produce chimeric antibodies can involve the following steps:

a) identifying and cloning the correct gene segment encoding the antigen binding portion of the antibody molecule; this gene segment (known as the VDJ, variable, diversity and joining regions for heavy chains or VJ, variable, joining regions for light chains or simply as the V or variable region) may be in either the cDNA or genomic form;

b) cloning the gene segments encoding the constant region or desired part thereof;

c) ligating the variable region with the constant region so that the complete chimeric antibody is encoded in a form that can be transcribed and translated;

d) ligating this construct into a vector containing a selectable marker and gene control regions such as promoters, enhancers and poly(A) addition signals;

e) amplifying this construct in bacteria;

f) introducing this DNA into eukaryotic cells (transfection) most often mammalian lymphocytes;

g) selecting for cells expressing the selectable marker;

h) screening for cells expressing the desired chimeric antibody; and

k) testing the antibody for appropriate binding specificity and effector functions.

Antibodies of several distinct antigen binding specificities have been manipulated by these protocols to produce chimeric proteins [e.g. anti-TNP: Boulianne et al., Nature 312:643 (1984); and anti-tumor antigens: Sahagan et al., J. Immunol. 137:1066 (1986)]. Likewise, several different effector functions have been achieved by linking new sequences to those encoding the antigen binding region. Some of these include enzymes [Neuberger et al., Nature 312:604 (1984)], immunoglobulin constant regions from another species and constant regions of another immunoglobulin chain [Sharon et al., Nature 309:364 (1984); Tan et al., J. Immunol. 135:3565-3567 (1985)]. Additionally, procedures for modifying antibody molecules and for producing chimeric antibody molecules using homologous recombination to target gene modification have been described (Fell et al., Proc. Natl. Acad. Sci. USA 86:8507-8511 (1989)).

These antibodies are capable of binding to PSCA, e.g., on the cell surface of prostate cancer cells, thereby confirming the cell surface localization of PSCA. Because these mAbs recognize epitopes on the exterior of the cell surface, they have utility for prostate cancer diagnosis and therapy. For example, these mAbs were used to locate sites of metastatic disease (Example 6). Another possibility is that they may be used (e.g., systemically) to target prostate cancer cells therapeutically when used alone or conjugated to a radioisotope or other toxin.

PSCA mAbs stain the cell surface in a punctate manner (see Example 5), suggesting that PSCA may be localized to specific regions of the cell surface. GPI-anchored proteins are known to cluster in detergent-insoluble glycolipid-enriched microdomains (DIGS) of the cell surface. These microdomains, which include caveolae and shingolipid-cholesterol rafts, are believed to

play critical roles in signal transduction and molecular transport. Thy-1, a homologue of PSCA, has previously been shown to transmit signals to src kinases through interaction in lipid-microdomains. Subcellular fractionation experiments in our laboratory confirm the presence of PSCA in DIGS.

Additionally, some of the antibodies of the invention are internalizing antibodies, i.e., the antibodies are internalized into the cell upon or after binding. It is intended that the term "internalize" means that the antibody is taken into the cell. Further, some of the antibodies induce inhibition of PSCA positive cancer cell growth.

A characterization of these antibodies, e.g., in prostate cancer specimens, demonstrates that PSCA protein is overexpressed in prostate cancers relative to normal cells and its expression appears to be upregulated during prostate cancer progression and metastasis. These antibodies are useful in studies of PSCA biology and function, as well as in vivo targeting of PSCA associated cancers, including, without limitation, human prostate cancer, prostate cancer metastases to bone, bladder carcinomas, and pancreatic carcinomas.

PSCA mAbs which specifically recognize and bind to the extracellular domain of the PSCA protein are described herein. Some of these have been shown to bind to native PSCA as expressed on the cell surface and some have been shown to inhibit the in vivo growth of prostate tumor cells.

The amino acid sequence of PSCA presented herein may be used to select specific regions of the PSCA protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the PSCA amino acid sequence may be used to identify hydrophilic regions in the PSCA structure. Regions of the PSCA protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Fragments containing these residues are particularly suited in generating specific classes of anti-PSCA antibodies.

Particularly useful fragments include, but are not limited to, the sequences TARIRAVGLLTVISK (SEQ ID NO. 16) and SLNCVDDSQDYYVGK (SEQ ID NO. 18).

As described in Example 2, below, a rabbit polyclonal antibody was generated against the former fragment, prepared as a synthetic peptide, and affinity purified using a PSCA-glutathione S transferase fusion protein. Recognition of PSCA by this antibody was established by immunoblot and immunoprecipitation analysis of extracts of 293T cells transfected with PSCA and a GST-PSCA fusion protein. This antibody also identified the cell surface expression of PSCA in PSCA-transfected 293T and LAPC-4 cells using fluorescence activated cell sorting (FACS).

Additionally, a sheep polyclonal antibody was generated against the latter fragment, prepared as a synthetic peptide, and affinity purified using a peptide Affi-gel column (also by the method of Example 2). Recognition of PSCA by this antibody was established by immunoblot and immunoprecipitation analysis of extracts of LNCaP cells transfected with PSCA. This antibody also identified the cell surface expression of PSCA in PSCA-transfected LNCaP cells using fluorescence activated cell sorting (FACS) and immunohistochemistry analysis.

Methods for preparing a protein for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be used; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be effective. Administration of a PSCA immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, monoclonal antibody preparations are preferred. Immortalized cell lines which secrete a desired monoclonal antibody may be

prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the PSCA protein or PSCA fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonal antibodies of the invention or the polyclonal antisera (e.g., Fab, $F(ab')_2$, Fv fragments, fusion proteins) which contain the immunologically significant portion (i.e., a portion that recognizes and binds PSCA) can be used as antagonists, as well as the intact antibodies. Humanized antibodies directed against PSCA is also useful. As used herein, a humanized PSCA antibody is an immunoglobulin molecule which is capable of binding to PSCA and which comprises a FR region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of non-human immunoglobulin or a sequence engineered to bind PSCA. Methods for humanizing murine and other non-human antibodies by substituting one or more of the non-human antibody CDRs for corresponding human antibody sequences are well known (see for example, Jones et al., 1986, Nature 321: 522-525; Riechmnan et al., 1988, Nature 332: 323-327; Verhoeyen et al., 1988, Science 239: 1534-1536). See also, Carter et al., 1993, Proc. Natl. Acad. Sci. USA 89: 4285 and Sims et al., 1993, J. Immunol. 151: 2296.

Use of immunologically reactive fragments, such as the Fab, Fab', or $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin. Further, bi-specific antibodies specific for two or more epitopes may be generated using methods generally known in the art. Further, antibody effector functions may be modified so as to enhance the therapeutic effect of PSCA antibodies on cancers. For example, cysteine residues may be engineered into the Fc region, permitting the formation of interchain disulfide bonds and the generation of homodimers which may have enhanced capacities for internalization, ADCC and/or

complement-mediated cell killing (see, for example, Caron et al., 1992, J. Exp. Med. 176: 1191-1195; Shopes, 1992, J. Immunol. 148: 2918-2922). Homodimeric antibodies may also be generated by cross-linking techniques known in the art (e.g., Wolff et al., Cancer Res. 53: 2560-2565). The invention also provides pharmaceutical compositions having the monoclonal antibodies or anti-idiotypic monoclonal antibodies of the invention.

The generation of monoclonal antibodies (mAbs) capable of binding to cell surface PSCA are described in Example 5. Epitope mapping of these mAbs indicates that they recognize different epitopes on the PSCA protein. For example, one recognizes an epitope within the carboxy-terminal region and the other recognizing an epitope within the amino-terminal region. Such PSCA antibodies may be particularly well suited to use in a sandwich-formatted ELISA, given their differing epitope binding characteristics.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the PSCA protein can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin. The invention includes an antibody, e.g., a monoclonal antibody which competitively inhibits the immunospecific binding of any of the monoclonal antibodies of the invention to PSCA.

Alternatively, methods for producing fully human monoclonal antibodies, include phage display and transgenic methods, are known and may be used for the generation of human mAbs (for review, see Vaughan et al., 1998, Nature Biotechnology 16: 535-539). For example, fully human anti-PSCA monoclonal antibodies may be generated using cloning technologies employing large human Ig gene combinatorial libraries (i.e., phage display)(Griffiths and Hoogenboom, Building an in vitro immune system: human antibodies from phage display libraries. In: Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man. Clark, M. (Ed.), Nottingham Academic, pp 45-64 (1993); Burton and Barbas, Human Antibodies from combinatorial libraries. Id., pp 65-82). Fully human anti-PSCA monoclonal antibodies may also be produced using transgenic mice engineered to contain human immunoglobulin gene loci as described in

PCT Patent Application WO98/24893, Jakobovits et al., published December 3, 1997 (see also, Jakobovits, 1998, Exp. Opin. Invest. Drugs 7(4): 607-614). This method avoids the in vitro manipulation required with phage display technology and efficiently produces high affinity authentic human antibodies.

Reactivity of anti-PSCA mAbs against the target antigen may be established by a number of well known means, including Western blot, immunoprecipitation, ELISA, and FACS analyses using, as appropriate, PSCA proteins, peptides, PSCA-expressing cells or extracts thereof. Anti-PSCA mAbs may also be characterized in various in vitro assays, including complement-mediated tumor cell lysis, antibody-dependent cell cytotoxicity (ADCC), antibody-dependent macrophage-mediated cytotoxicity (ADMMC), tumor cell proliferation, etc. Examples of such in vitro assays are presented in Example 19, infra.

The antibody or fragment thereof of the invention may be cytostatic to the cell, to which it binds. It is intended that the term "cytostatic" means that the antibody can inhibit growth, but not necessarily kill, PSCA-positive cells.

The antibody or fragment thereof of the invention may be labeled with a detectable marker or conjugated to a second molecule, such as a therapeutic agent (e.g., a cytotoxic agent) thereby resulting in an immunoconjugate. For example, the therapeutic agent includes, but is not limited to, an anti-tumor drug, a toxin, a radioactive agent, a cytokine, a second antibody or an enzyme. Further, the invention provides an embodiment wherein the antibody of the invention is linked to an enzyme that converts a prodrug into a cytotoxic drug.

The immunoconjugate can be used for targeting the second molecule to a PSCA positive cell (Vitetta, E.S. et al., 1993, Immunotoxin therapy, in DeVita, Jr., V.T. et al., eds, Cancer: Principles and Practice of Oncology, 4th ed., J.B. Lippincott Co., Philadelphia, 2624-2636).

Examples of cytotoxic agents include, but are not limited to ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, *Pseudomonas* exotoxin (PE) A, PE40, abrin, arbrin A chain, modeccin A chain, alpha-sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Antibodies may also be conjugated to an anti-cancer pro-drug activating enzyme capable of converting the pro-drug to its active form. See, for example, U.S. Patent No. 4,975,287.

Additionally, the recombinant protein of the invention comprising the antigen-binding region of any of the monoclonal antibodies of the invention can be used to treat cancer. In such a situation, the antigen-binding region of the recombinant protein is joined to at least a functionally active portion of a second protein having therapeutic activity. The second protein can include, but is not limited to, an enzyme, lymphokine, oncostatin or toxin. Suitable toxins include those described above.

Techniques for conjugating or joining therapeutic agents to antibodies are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)). The use of PSCA antibodies as therapeutic agents is further described in the subsection "PROSTATE CANCER IMMUNOTHERAPY" below.

PSCA-ENCODING NUCLEIC ACID MOLECULES

Another aspect of the invention provides various nucleic acid molecules encoding PSCA proteins and fragments thereof, preferably in isolated form, including DNA, RNA, DNA/RNA hybrid, and related molecules, nucleic acid molecules complementary to the PSCA coding sequence or a part thereof, and those which hybridize to the PSCA gene or to PSCA-encoding nucleic acids. Particularly preferred nucleic acid molecules will have a nucleotide sequence substantially identical to or complementary to the human or murine DNA sequences herein disclosed. Specifically contemplated are genomic DNA, cDNAs, ribozymes, and antisense molecules, as well as nucleic acids based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized.

For example, antisense molecules can be RNAs or other molecules, including peptide nucleic acids (PNAs) or non-nucleic acid molecules such as phosphorothioate derivatives, that specifically bind DNA or RNA in a base pair-dependent manner. A skilled artisan can readily obtain these classes of nucleic acid molecules using the herein described *PSCA* sequences. For convenience, PSCA-encoding nucleic acid molecules will be referred to herein as PSCA-encoding nucleic acid molecules, PSCA genes, or PSCA sequences.

The nucleotide sequence of a cDNA (SEQ ID NO:1) encoding one allelic form of human PSCA is provided in FIG. 1A. The nucleotide sequence of a cDNA (SEQ ID NO:3) encoding a murine PSCA homologue ("murine PSCA") is provided in FIG. 2. Genomic clones of human and murine PSCA have also been isolated, as described in Example 4. Both the human and murine genomic clones contain three exons encoding the translated and 3' untranslated regions of the PSCA gene. A fourth exon encoding a 5' untranslated region is presumed to exist based on PSCA's homology to other members of the Ly-6 and Thy-1 gene families (FIG. 8).

The human PSCA gene maps to chromosome 8q24.2. Human stem cell antigen 2 (RIG-E), as well as one other recently identified human Ly-6 homologue (E48) are also localized to this region, suggesting that a large family of related genes may exist at this locus (Brakenhoff et al., 1995, supra; Mao et al., 1996, Proc. Natl. Acad. Sci. USA 93: 5910-5914). Intriguingly, chromosome 8q has been reported to be a region of allelic gain and amplification in a majority of advanced and recurrent prostate cancers (Cher et al., 1994, Genes Chrom. Cancer 11: 153-162). c-myc localizes proximal to PSCA at chromosome 8q24 and extra copies of c-myc (either through allelic gain or amplification) have been found in 68% of primary prostate tumors and 96% of metastases (Jenkins et al., 1997, Cancer Res. 57: 524-531).

Embodiments of the PSCA-encoding nucleic acid molecules of the invention include primers, which allow the specific amplification of nucleic acid molecules of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. The nucleic acid probes can be labeled with a detectable marker. Examples of a detectable marker include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator or an enzyme. Such labeled probes can be used to diagnosis the presence of a PSCA protein as a means for diagnosing cell expressing a PSCA protein. Technologies for generating DNA and RNA probes are well known.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules that encode polypeptides other than PSCA. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated PSCA-encoding nucleic acid molecule.

The invention further provides fragments of the PSCA-encoding nucleic acid molecules of the present invention. As used herein, a fragment of a PSCA-encoding nucleic acid molecule refers to a small portion of the entire PSCA-encoding sequence. The size of the fragment will be determined by its intended use.

For example, if the fragment is chosen so as to encode an active portion of the PSCA protein, such an active domain, effector binding site or GPI binding domain, then the fragment will need to be large enough to encode the functional region(s) of the PSCA protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming.

Fragments of human *PSCA* that are particularly useful as selective hybridization probes or PCR primers can be readily identified from the entire *PSCA* sequence using art-known methods. One set of PCR primers that are useful for RT-PCR analysis comprise 5' - TGCTTGCCCTGTTGATGGCAG — (SEQ ID NO. 19) and 3' - CCAGAGCAGCAGGCCGAGTGCA — (SEQ ID NO. 20).

METHODS FOR ISOLATING OTHER PSCA-ENCODING NUCLEIC ACID MOLECULES

The PSCA-encoding nucleic acid molecules described herein enable the isolation of PSCA homologues, alternatively sliced isoforms, allelic variants, and mutant forms of the PSCA protein as well as their coding and gene sequences. The most preferred source of PSCA homologs are mammalian organisms.

For example, a portion of the PSCA-encoding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the PSCA family of proteins from organisms other than human, allelic variants of the human PSCA protein herein described, and genomic sequence containing the *PSCA* gene. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives. In a particular embodiment, cDNA encoding human PSCA was used to isolate a full length cDNA encoding the murine PSCA homologue as described in Example 3 herein. The murine clone encodes a protein with 70% amino acid identity to human PSCA.

In addition, the amino acid sequence of the human PSCA protein may be used to generate antibody probes to screen expression libraries prepared from cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe an expression library, prepared from a target organism, to obtain the appropriate coding sequence for a PSCA homologue. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructing an expression cassette using control sequences appropriate to the particular host used for expression of the enzyme.

Genomic clones containing PSCA genes may be obtained using molecular cloning methods well known in the art. In one embodiment, a human genomic clone of approximately 14kb containing exons 1-4 of the PSCA gene was obtained by screening a lambda phage library with a human PSCA cDNA probe, as more completely described in Example 4 herein. In another embodiment, a genomic clone of approximately 10kb containing the murine PSCA gene was obtained by screening a murine BAC (bacterial artificial chromosome) library with a murine PSCA cDNA (also described in Example 4).

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively amplify/clone a PSCA-encoding nucleic acid molecule, or fragment thereof. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other PSCA-encoding nucleic acid molecules. Regions of the human PSCA gene that are particularly well suited for use as a probe or as primers can be readily identified.

Non-human homologues of *PSCA*, naturally occurring allelic variants of *PSCA* and genomic *PSCA* sequences will share a high degree of homology to the human *PSCA* sequences herein described. In general, such nucleic acid molecules will hybridize to the human *PSCA* sequence under stringent conditions. Such sequences will typically contain at least 70% homology, preferably at least 80%, most preferably at least 90% homology to the human *PSCA* sequence.

Stringent conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium nitrate/0.1% SDS at 50°C., or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C.

Another example is use of 50% formamide, 5 x SSC (0.75M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2 x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

RECOMBINANT DNA MOLECULES CONTAINING PSCA-ENCODING NUCLEIC ACIDS

Also provided are recombinant DNA molecules (rDNAs) that contain a PSCA-encoding sequences as herein described, or a fragment thereof. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in vitro*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al.*, *Molecular Cloning* (1989). In the preferred rDNA molecules of the present invention, a PSCA-encoding DNA sequence that encodes a PSCA protein or a fragment of PSCA, is operably linked to one or more expression control sequences and/or vector sequences. The rDNA molecule can encode either the entire PSCA protein, or can encode a fragment of the PSCA protein.

The choice of vector and/or expression control sequences to which the PSCA-encoding sequence is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or

insertion into the host chromosome, and preferably also expression, of the PSCA-encoding sequence included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, enhancers, transcription terminators and other regulatory elements. Preferably, an inducible promoter that is readily controlled, such as being responsive to a nutrient in the host cell's medium, is used.

In one embodiment, the vector containing a PSCA-encoding nucleic acid molecule will include a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule intrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or viral promoter capable of directing the expression (transcription and translation) of the PSCA-encoding sequence in a bacterial host cell, such as *E. coli.* A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Various viral vectors well known to those skilled in the art may also be used, such as, for example, a number of well known retroviral vectors.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to variant rDNA molecules that contain a PSCA-encoding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing

convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are PSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (*neo*) gene. Southern *et al.*, *J Mol Anal Genet* (1982) 1:327-341. Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by cotransfection of the host cell, and selected by culturing in the presence of the appropriate drug for the selectable marker.

In accordance with the practice of the invention, the vector can be a plasmid, cosmid or phage vector encoding the cDNA molecule discussed above. Additionally, the invention provides a host-vector system comprising the plasmid, cosmid or phage vector transfected into a suitable eukaryotic host cell. Examples of suitable eukaryotic host cells include a yeast cell, a plant cell, or an animal cell, such as a mammalian cell. Examples of suitable cells include the LnCaP, LAPC-4, and other prostate cancer cell lines. The host-vector system is useful for the production of a PSCA protein. Alternatively, the host cell can be prokaryotic, such as a bacterial cell.

TRANSFORMED HOST CELLS

The invention further provides host cells transformed with a nucleic acid molecule that encodes a PSCA protein or a fragment thereof. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a PSCA protein are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of a PSCA gene. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably

vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Prostate cancer cell lines, such as the LnCaP and LAPC-4 cell lines may also be used. Any prokaryotic host can be used to express a PSCA-encoding rDNA molecule. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with an rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen *et al.*, *Proc Acad Sci USA* (1972) 69:2110; and Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed, see, for example, Graham *et al.*, *Virol* (1973) 52:456; Wigler *et al.*, *Proc Natl Acad Sci USA* (1979) 76:1373-76.

Successfully transformed cells, i.e., cells that contain an rDNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J Mol Biol* (1975) 98:503, or Berent *et al.*, *Biotech* (1985) 3:208 or the proteins produced from the cell assayed via an immunological method.

RECOMBINANT METHODS OF GENERATING PSCA PROTEINS

The invention further provides methods for producing a PSCA protein using one of the PSCA-encoding nucleic acid molecules herein described. In general terms, the production of a recombinant PSCA protein typically can involve the following steps (Maniatis, supra).

First, a nucleic acid molecule is obtained that encodes a PSCA protein (SEQ ID NO: 2 or 4) or a fragment thereof, such as the nucleic acid molecule depicted in FIG. 1A. The PSCA-

encoding nucleic acid molecule is then preferably placed in an operable linkage with suitable control sequences, as described above, to generate an expression unit containing the PSCA-encoding sequence. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the PSCA protein. Optionally the PSCA protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps may be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in an appropriate host. The construction of expression vectors that are operable in a variety of hosts is accomplished using an appropriate combination of replicons and control sequences. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with PSCA-encoding sequences to produce a PSCA protein.

In a specific embodiment described in the examples which follow, a secreted form of PSCA may be conveniently expressed in 293T cells transfected with a CMV-driven expression vector encoding PSCA with a C-terminal 6XHis and MYC tag (pcDNA3.1/mycHIS, Invitrogen). The secreted HIS-tagged PSCA in the culture media may be purified using a nickel column using standard techniques.

ASSAYS FOR IDENTIFYING PSCA LIGANDS AND OTHER BINDING AGENTS

Another aspect of the invention relates to assays and methods that can be used to detect and identify PSCA ligands and other agents and cellular constituents that bind to PSCA. Specifically, PSCA ligands and other agents and cellular constituents that bind to PSCA can be identified by the ability of the PSCA ligand or other agent or constituent to bind to PSCA

and/or the ability to inhibit/stimulate PSCA activity. Assays for PSCA activity (e.g., binding) using a PSCA protein are suitable for use in high through-put screening methods.

In one embodiment, the assay comprises mixing PSCA with a test agent or cellular extract. After mixing under conditions that allow association of PSCA with the agent or component of the extract, the mixture is analyzed to determine if the agent/component is bound to PSCA. Binding agents/components are identified as being able to bind to PSCA. Alternatively or consecutively, PSCA activity can be directly assessed as a means for identifying agonists and antagonists of PSCA activity.

Alternatively, targets that bind to a PSCA protein can be identified using a yeast two-hybrid system (Fields, S. and Song, O. (1989), Nature 340:245-246) or using a binding-capture assay (Harlow, supra). In the yeast two hybrid system, an expression unit encoding a fusion protein made up of one subunit of a two subunit transcription factor and the PSCA protein is introduced and expressed in a yeast cell. The cell is further modified to contain (1) an expression unit encoding a detectable marker whose expression requires the two subunit transcription factor for expression and (2) an expression unit that encodes a fusion protein made up of the second subunit of the transcription factor and a cloned segment of DNA. If the cloned segment of DNA encodes a protein that binds to the PSCA protein, the expression results in the interaction of the PSCA and the encoded protein. This brings the two subunits of the transcription factor into binding proximity, allowing reconstitution of the transcription factor. This results in the expression of the detectable marker. The yeast two hybrid system is particularly useful in screening a library of cDNA encoding segments for cellular binding partners of PSCA.

PSCA proteins which may be used in the above assays include, but are not limited to, an isolated PSCA protein, a fragment of a PSCA protein, a cell that has been altered to express a PSCA protein, or a fraction of a cell that has been altered to express a PSCA protein. Further, the PSCA protein can be the entire PSCA protein or a defined fragment of the PSCA protein. It will be apparent to one of ordinary skill in the art that so long as the PSCA

protein can be assayed for agent binding, e.g., by a shift in molecular weight or activity, the present assay can be used.

The method used to identify whether an agent/cellular component binds to a PSCA protein will be based primarily on the nature of the PSCA protein used. For example, a gel retardation assay can be used to determine whether an agent binds to PSCA or a fragment thereof. Alternatively, immunodetection and biochip technologies can be adopted for use with the PSCA protein. A skilled artisan can readily employ numerous art-known techniques for determining whether a particular agent binds to a PSCA protein.

Agents and cellular components can be further tested for the ability to modulate the activity of a PSCA protein using a cell-free assay system or a cellular assay system. As the activities of the PSCA protein become more defined, functional assays based on the identified activity can be employed.

As used herein, an agent is said to antagonize PSCA activity when the agent reduces PSCA activity. The preferred antagonist will selectively antagonize PSCA, not affecting any other cellular proteins. Further, the preferred antagonist will reduce PSCA activity by more than 50%, more preferably by more than 90%, most preferably eliminating all PSCA activity.

As used herein, an agent is said to agonize PSCA activity when the agent increases PSCA activity. The preferred agonist will selectively agonize PSCA, not affecting any other cellular proteins. Further, the preferred antagonist will increase PSCA activity by more than 50%, more preferably by more than 90%, most preferably more than doubling PSCA activity.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences of the PSCA protein. An example of randomly selected agents is the use of a chemical library or a peptide combinatorial library, or a growth broth of an organism or plant extract.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis that takes into account the sequence of the target site and/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the PSCA protein. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to a fragment of a PSCA protein.

The agents tested in the methods of the present invention can be, as examples, peptides, small molecules, and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents used in the present screening method. One class of agents of the present invention are peptide agents whose amino acid sequences are chosen based on the amino acid sequence of the PSCA protein. Small peptide agents can serve as competitive inhibitors of PSCA protein assembly.

Peptide agents can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with critical positions of the PSCA protein. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the PSCA protein intended to be targeted by the antibodies. Critical regions may include the domains identified in FIG. 15. Such agents can be used in competitive binding studies to identify second generation inhibitory agents.

The cellular extracts tested in the methods of the present invention can be, as examples, aqueous extracts of cells or tissues, organic extracts of cells or tissues or partially purified cellular fractions. A skilled artisan can readily recognize that there is no limit as to the source of the cellular extract used in the screening method of the present invention.

Agents that bind a PSCA protein, such as a PSCA antibody, can be used to modulate the activity of PSCA, to target anticancer agents to appropriate mammalian cells, or to identify agents that block the interaction with PSCA. Cells expressing PSCA can be targeted or identified by using an agent that binds to PSCA.

How the PSCA binding agents will be used depends on the nature of the PSCA binding agent. For example, a PSCA binding agent can be used to: deliver conjugated toxins, such a diphtheria toxin, cholera toxin, ricin or pseudomonas exotoxin, to a PSCA expressing cell; modulate PSCA activity; to directly kill PSCA expressing cells; or in screens to identify competitive binding agents. For example, a PSCA inhibitory agent can be used to directly inhibit the growth of PSCA expressing cells whereas a PSCA binding agent can be used as a diagnostic agent.

There are multiple diagnostic uses of the invention. For example, the invention provides methods for diagnosing in a subject, e.g., an animal or human subject, a cancer associated with the presence of the PSCA protein. In one embodiment, the method comprises quantitatively determining the number of PSCA protein in the sample (e.g., cell or biological fluid sample) using any one or combination of the antibodies of the invention. Then the number so determined can be compared with the amount in a sample from a normal subject. The presence of a measurably different amount (i.e., the number of PSCA in the test sample exceeds the number from a normal sample) in the samples indicating the presence of the cancer. PSCA is overexpressed on a cell when the number of PSCA in the test sample exceeds the number from a normal sample.

In another embodiment, diagnosis involves quantitatively determining in a sample from the subject the amount of RNA encoding the PSCA protein using the nucleic acid of the

invention. The amount so determined can be compared with the amount of RNA in a sample from a normal subject. Once again, the presence of a measurable different amount indicating the presence of the cancer.

Additionally, the invention provides methods for monitoring the course of cancer (e.g., prostate, bone metastases of prostate cancer, bladder, pancreatic cancer) or disorders associated with PSCA in a subject by measuring the amount of PSCA in a sample from the subject at various points in time. This is done for purposes of determining a change in the amount of PSCA in the sample e.g., to determine whether the change is a small change in the amount or a large change, i.e., overexpression of PSCA. In one embodiment, the method comprises quantitatively determining in a first sample from the subject the presence of a PSCA protein and comparing the amount so determined with the amount present in a second sample from the subject, such samples being taken at different points in time, a difference in the amounts determined being indicative of the course of the cancer.

In another embodiment, monitoring is effected by quantitatively determining in a first sample from the subject the presence of a PSCA RNA and comparing the amount so determined with the amount present in a second sample from the subject, such samples being taken at different points in time, a difference in the amounts determined being indicative of the course of the cancer (e.g, prostate, bone metastases of prostate cancer, bladder and pancreatic cancer).

As a further embodiment, the diseases or disorders associated with PSCA can be monitored in a sample by detecting an increase in or increased PSCA gene copy number. An increase in or increased PSCA gene copy number is important because it may correlate with poor outcome.

The sample can be from an animal or a human. Further, the sample can be a cell sample. For example, using the methods of the invention, organ tissues such as prostate tissue, bladder tissue, pancreatic tissue, neuroendocrine tissue, and bone (any tissue where

carcinomas can metastasize, e.g., node, lung, liver, pancreas) can be evaluated for the presence of cancer or metastatic lesion. Alternatively, the sample can be a biological fluid, e.g., urine, blood sera or plasma.

In accordance with the practice of the invention, detection can be effected by immunologic detection means involving histology, blotting, ELISA, and ELIFA. When the sample is a tissue or cell sample it can be formalin-fixed, paraffin-embedded or frozen.

The invention additionally provides methods of determining a difference in the amount and distribution of PSCA in tissue sections from a neoplastic tissue to be tested relative to the amount and distribution of PSCA in tissue sections from a normal tissue. In one embodiment, the method comprises contacting both the tissue to be tested and the normal tissue with a monoclonal antibody that specifically forms a complex with PSCA and thereby detecting the difference in the amount and distribution of PSCA.

Further, the invention provides a method for diagnosing a neoplastic or preneoplastic condition in a subject. This method comprises obtaining from the subject a sample of a tissue, detecting a difference in the amount and distribution of PSCA in the using the method above, a distinct measurable difference being indicative of such neoplastic or preneoplastic condition.

In accordance with the practice of the invention, the antibody can be directed to the epitope to which any of the monoclonal antibodies of the invention is directed. Further, the tissue section can be from the bladder, prostate, bone, lymphatic tissues, pancreas, other organs, or muscle.

The invention also provides methods of detecting and quantitatively determining the concentration of PSCA in a biological fluid sample. In one embodiment the method comprises contacting a solid support with an excess of one or more monoclonal antibodies which forms (preferably specifically forms) a complex with PSCA under conditions permitting the monoclonal antibody to attach to the surface of the solid

support. The resulting solid support to which the monoclonal antibody is attached is then contacted with a biological fluid sample so that the PSCA in the biological fluid binds to the antibody and forms a PSCA-antibody complex. The complexed can be labeled directly or indirectly with a detectable marker. Alternatively, either the PSCA or the antibody can be labeled before the formation the complex. The complex can then be detected and quantitatively determined thereby detecting and quantitatively determining the concentration of PSCA in the biological fluid sample. A high concentration of PSCA in the sample relative to normal cells being indicative of a neoplastic or preneoplastic condition.

In accordance with the practice of the invention, the biological fluid includes but is not limited to tissue extract, urine, blood, serum, and phlegm. Further, the detectable marker includes but is not limited to an enzyme, biotin, a fluorophore, a chromophore, a heavy metal, a paramagnetic isotope, or a radioisotope.

Further, the invention provides a diagnostic kit comprising an antibody that recognizes and binds PSCA (an anti-PSCA antibody); and a conjugate of a detectable label and a specific binding partner of the anti-PSCA antibody. In accordance with the practice of the invention the label includes, but is not limited to, enzymes, radiolabels, chromophores and fluorescers.

CANCER IMMUNOTHERAPY

Since PSCA protein is expressed or overexpressed in many cancers, including but not limited to prostate tumors, metastases of prostate tumors (such as bone metastases), bladder cancer and pancreatic cancer, it is a target for cancer immunotherapy. These immunotherapeutic methods include the use of antibody therapy, in vivo vaccines, and ex vivo immunotherapy approaches.

In one approach, the invention provides PSCA antibodies that may be used systemically to treat cancer, such as prostate, bladder and pancreatic cancer. PSCA antibodies may also be useful in the treatment of various other benign and malignant tumors. Antibodies which

bind specifically to the extracellular domain of PSCA are preferred. Antibodies which target the tumor cells but not the surrounding non-tumor cells and tissue are preferred. Thus, the invention provides a method of treating a patient susceptible to or having a cancer which expresses PSCA antigen, comprising administering to said patient an effective amount of an antibody which binds specifically to the extracellular domain of PSCA. In another approach, the invention provides a method of inhibiting the growth of tumor cells expressing PSCA, comprising administering to a patient an antibody which binds specifically to the extracellular domain of PSCA in an amount effective to inhibit growth of the tumor cells. PSCA mAbs may also be used in a method for selectively inhibiting the growth of or killing a cell expressing PSCA antigen comprising reacting a PSCA antibody immunoconjugate or immunotoxin with the cell in an amount sufficient to inhibit the growth of or kill the cell.

For example, unconjugated PSCA antibody (including monoclonal, polyclonal, chimeric, humanized, fully human and fragments thereof (e.g., recombinant proteins)) may be introduced into a patient such that the antibody binds to PSCA on cancer cells and mediates growth inhibition of such cells (including the destruction thereof), and the tumor, by mechanisms which may include complement-mediated cytolysis, antibody-dependent cellular cytotoxicity, altering the physiologic function of PSCA, and/or the inhibition of ligand binding or signal transduction pathways. In addition to unconjugated PSCA antibodies, fragments thereof, and recombinant proteins of the invention, PSCA antibodies conjugated to toxic agents such as ricin may also be used therapeutically to deliver the toxic agent directly to PSCA-bearing tumor cells and thereby destroy the tumor.

Cancer immunotherapy using PSCA antibodies may follow the teachings generated from various approaches which have been successfully employed with respect to other types of cancer, including but not limited to colon cancer (Arlen et al., 1998, Crit Rev Immunol 18: 133-138), multiple myeloma (Ozaki et al., 1997, Blood 90: 3179-3186; Tsunenari et al., 1997, Blood 90: 2437-2444), gastric cancer (Kasprzyk et al., 1992, Cancer Res 52: 2771-2776), B-cell lymphoma (Funakoshi et al., 1996, J Immunther Emphasis Tumor Immunol 19: 93-101), leukemia (Zhong et al., 1996, Leuk Res 20: 581-589), colorectal cancer (Moun

et al., 1994, Cancer Res 54: 6160-6166); Velders et al., 1995, Cancer Res 55: 4398-4403), and breast cancer (Shepard et al., 1991, J Clin Immunol 11: 117-127).

For example, one way to apply antitumor monoclonal antibodies clinically is to administer them in unmodified form, using monoclonal antibodies of the invention which display antitumor activity (e.g., ADCC and CDC activity) and/or internalizing ability in vitro and/or in animal models (see, e.g. Hellstrom et al., Proc. Natl. Acad. Sci. USA 82:1499-1502 (1985). To detect ADCC and CDC activity monoclonal antibodies can be tested for lysing cultured ^{51}Cr-labeled tumor target cells over a 4-hour incubation period. Target cells are labeled with ^{51}Cr and then can be exposed for 4 hours to a combination of effector cells (in the form of human lymphocytes purified by the use of a lymphocyte-separation medium) and antibody, which is added in concentrations, e.g., varying between 0.1 μg/ml and 10 μg/ml. The release of ^{51}Cr from the target cells is measured as evidence of tumor-cell lysis (cytotoxicity). Controls include the incubation of target cells alone or with either lymphocytes or monoclonal antibody separately. The total amount of ^{51}Cr that can be released is measured and ADCC is calculated as the percent killing of target cells observed with monoclonal antibody plus effector cells as compared to target cells being incubated alone. The procedure for CDC is identical to the one used to detect ADCC except that human serum, as a source of complement, (diluted 1:3 to 1:6) is added in place of the effector cells.

In the practice of the method of the invention, anti-PSCA antibodies capable of inhibiting the growth of cancer cells expressing PSCA on the cell surface are administered in a therapeutically effective amount to cancer patients whose tumors express or overexpress PSCA. The anti-PSCA mAb therapy method of the invention demonstrates remarkable tumor growth inhibition of prostate tumors in vivo. Accordingly, the invention provides a specific, effective and long-needed treatment for prostate cancer. The method of the invention may also be useful for the treatment of other cancers which express or overexpress PSCA, including but not limited to bladder carcinoma and pancreatic carcinomas, since both of these cancers express elevated levels of PSCA. The antibody therapy methods of the

invention may be combined with a chemotherapeutic, radiation, and/or other therapeutic regimen.

As described in Example 18A below, individual mouse anti-PSCA mAbs, as well as combinations of these anti-PSCA monoclonal antibodies, are capable of significantly inhibiting prostate tumor growth in vivo using a xenogenic prostate cancer SCID mouse model. In one study, a cohort of SCID mice receiving injections of a human prostate tumor xenograft were treated with a combination of several murine anti-PSCA mAbs. The results of this study showed that the treatment was able to completely block the formation of tumors in all of these mice - even after 61 days post tumor injection. In contrast, all animals in a control group of SCID mice receiving the same prostate tumor xenograft, but treated with control murine IgG, developed significant and progressively more massive tumors during the study. There was no apparent toxicity associated with the treatment of these animals with the anti-PSCA mAb preparation, as all mice in the treatment group remained lively and healthy throughout the experiment. The xenograft used in the study, LAPC-9, was generated from a bone tumor biopsy of a patient with hormone-refractory metastatic prostate cancer, is characterized by an extremely androgen-sensitive phenotype (PSA levels drop to zero after castration in recipient SCID mice), particularly aggressive growth properties, and high level overexpression of PSCA. LAPC-9 and is described further elsewhere (Published PCT Application WO98/16628, Sawyers et al., April 23, 1998). These results were confirmed in a second in vivo study described in Example 18B. In addition, further in vivo studies demonstrated that anti-PSCA mAbs are therapeutically effective when used alone (Example 18C1, C2). In all of these in vivo studies, tumors in mice receiving the anti-PSCA mAb treatments had significantly slower growth rates, longer latency periods, and were smaller in size compared to tumors in mice receiving control antibody treatments. Serum PSA levels were also lower in relation to control treated animals and correlated with tumor inhibition. Moreover, antibodies recognizing different PSCA epitopes, as well as antibodies having different IgG isotypes, are therapeutically effective. In one study, anti-PSCA mAbs effectively inhibited the growth of established prostate tumors in vivo (Example 18, C4). Some of the mice treated in this particular study showed tumor regression following PSCA treatment (Example 18).

Additionally, the 3C5 antibody, administered to a tumor-bearing mouse, targeted the tumor cells that express PSCA. A SCID mouse bearing an LAPC-9 tumor (e.g., expressed PSCA), was treated with 3C5 antibody. The tumor was explanted and examined for the presence of the 3C5 antibody, by immunohistochemistry analysis (Example 26, Figure 71). The fixed tissue slices were probed with goat anti-mouse IgG. The 3C5 antibody was localized to the mass of PSCA-expressing tumor cells (Figure 71) and could be detected throughout the tumor. Because SCID mice produce no immunoglobulin, the antibody detected in the tumor tissue most likely originated from the 3C5 treatment. To confirm the localization of the 3C5 antibody, Western blot analysis was performed on tumor explants from the same mouse. The blot included protein extracts from the tumor explant, control IgG antibody, and 3C5 antibody, and the blot was probed with goat anti-mouse IgG-HRP antibodies. The IgG heavy and light chains were readily detected in the tumor lysates from the 3C5-treated mouse (Figure 72).

The results of a different study also indicate that anti-PSCA antibodies can target PSCA-expressing tumors. A SCID mouse bearing an established LAPC-9 tumor was treated with 1G8 antibody. The explanted tumor was examined for the presence of the 1G8 antibody, by Western blot analysis (Example 26, Figure 72) using goat anti-mouse IgG-HRP antibodies as a probe. The heavy and light chains were readily detectable in the 1G8-treated mouse. These results indicate that anti-PSCA antibodies administered to an subject, can circulate and target a PSCA-expressing tumor. This suggests that anti-PSCA monoclonal antibodies can circulate and target PSCA-expressing cells in tumors that are local, locally recurring, and metastatic. Furthermore, this suggests that conjugated anti-PSCA monoclonal antibodies can target and kill tumors cells expressing PSCA.

As described in Example 24 below, individual anti-PSCA mAbs are capable of inhibiting prostate tumor growth in vivo, in a xenogenic prostate cancer SCID mouse model. For example, two cohorts of SCID mice received injections of LAPC-9, and were treated with 1G8 or 3C5. The results showed that treatment with 1G8 or 3C5 alone inhibited tumor growth in the tumor-bearing mice. In contrast, the mice in a control group that received the

same prostate tumor xenograft, but treated with murine IgG or phosphate buffer, developed larger tumors during the study. In addition, the anti-PSCA treatment significantly prolonged the life of the mice receiving the antibody treatment, compared to the control mice. The prolonged life of the antibody-treated mice correlated with a decrease in tumor growth, and effected the level of serum PSA levels. These results indicate that treatment with anti-PSCA antibody can prolong the life of a tumor-bearing animal, by inhibiting tumor growth.

The effect of anti-PSCA mAbs in combination with an cytotoxic agent was also tested. As described in Example 25 below, two cohorts of SCID mice received injections of PC3 cells which were engineered to express PSCA, and the mice were treated with 1G8 alone or in combination with doxorubicin. The results showed that treatment with 1G8 inhibited tumor growth of the PSCA-positive PC3 cells, and the combination of 1G8 and doxorubicin had a synergetic effect on inhibiting tumor growth, compared to the tumors in mice treated with phosphate buffer or doxorubicin alone.

Thus, the results of Example 24 show that anti-PSCA monoclonal antibodies, having different isotypes, are effective in inhibiting the growth of established androgen-dependent tumors. For example, the LAPC-9 xenograft was generated from a bone tumor biopsy of a patient with hormone-refractory metastatic prostate cancer. The 1G8 antibody is a mouse gamma-1, isotypic, neutral antibody, which interacts directly with the PSCA antigen. The 3C5 antibody is a mouse gamma-2A isotypic, antibody, which binds to cells and complement. Thus, the 1G8 antibody may direct cell cytotoxicity of androgen-dependent tumors, through an antibody-dependent cell cytotoxicity (ADCC) mechanism, and the 3C5 antibody may initiate a potent immune response against the tumor. In a similar manner, the results of Example 25 show that anti-PSCA antibodies are effective in treating established androgen-independent tumors.

Patients may be evaluated for the presence and level of PSCA overexpression in tumors, preferably using immunohistochemical assessments of tumor tissue, quantitative PSCA imaging, or other techniques capable of reliably indicating the presence and degree of PSCA expression. Immunohistochemical analysis of tumor biopsies or surgical specimens may be

preferred for this purpose. Methods for immunohistochemical analysis of tumor tissues are well known in the art. An example of an immunohistochemical analytical technique useful for determining the level of PSCA overexpression in a sample is described in the example sections below.

Anti-PSCA monoclonal antibodies useful in treating cancer include those which are capable of initiating a potent immune response against the tumor and those which are capable of direct cytotoxicity. In this regard, anti-PSCA mAbs may elicit tumor cell lysis by either complement-mediated or antibody-dependent cell cytotoxicity (ADCC) mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins. In addition, anti-PSCA mAbs which exert a direct biological effect on tumor growth are useful in the practice of the invention. Such mAbs may not require the complete immunoglobulin to exert the effect. Potential mechanisms by which such directly cytotoxic mAbs may act include inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism by which a particular anti-PSCA mAb exerts an anti-tumor effect may be evaluated using any number of in vitro assays designed to determine ADCC, ADMMC, complement-mediated cell lysis, and so forth, such as those described in Example 19, below.

The anti-tumor activity of a particular anti-PSCA mAb, or combination of anti-PSCA mAbs, is preferably evaluated in vivo using a suitable animal model. Xenogenic cancer models, wherein human cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice, are particularly appropriate and are known. Examples of xenograft models of human prostate cancer (capable of recapitulating the development of primary tumors, micrometastasis, and the formation of osteoblastic metastases characteristic of late stage disease) are described in Klein et al., 1997, Nature Medicine 3: 402-408 and in PCT Patent Application WO98/16628, Sawyers et al., published April 23, 1998. The examples herein provide detailed experimental protocols for evaluating the anti-tumor potential of anti-PSCA mAb preparations in vivo.

Other in vivo assays are contemplated, such as those which measure regression of established tumors, interference with the development of metastasis, and the like.

It should be noted that the use of murine or other non-human monoclonal antibodies and chimeric mAbs may induce moderate to strong immune responses in some patients. In the most severe cases, such an immune response may lead to the extensive formation of immune complexes which, potentially, can cause renal failure. Accordingly, preferred monoclonal antibodies used in the practice of the therapeutic methods of the invention are those which are either fully human or humanized and which bind specifically to the target PSCA antigen with high affinity but exhibit low or no antigenicity in the patient.

The method of the invention contemplate the administration of single anti-PSCA mAbs as well as combinations, or "cocktails, of different individual mAbs such as those recognizing different epitopes. Such mAb cocktails may have certain advantages inasmuch as they contain mAbs which bind to different epitopes and/or exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination may exhibit synergistic therapeutic effects. In addition, the administration of anti-PSCA mAbs may be combined with other therapeutic agents, including but not limited to various chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL-2, GM-CSF). The anti-PSCA mAbs may be administered in their "naked" or unconjugated form, or may have therapeutic agents conjugated to them.

The anti-PSCA monoclonal antibodies used in the practice of the method of the invention may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the anti-PSCA mAbs retains the anti-tumor function of the antibody and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

The anti-PSCA antibody formulations may be administered via any route capable of delivering the antibodies to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. The preferred route of administration is by intravenous injection. A preferred formulation for intravenous injection comprises the anti-PSCA mAbs in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or diluted in polyvinylchloride or polyethylene bags containing 0.9% sterile Sodium Chloride for Injection, USP. The anti-PSCA mAb preparation may be lyophilized and stored as a sterile powder, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

Treatment will generally involve the repeated administration of the anti-PSCA antibody preparation via an acceptable route of administration such as intravenous injection (IV), at an effective dose. Dosages will depend upon various factors generally appreciated by those of skill in the art, including without limitation the type of cancer and the severity, grade, or stage of the cancer, the binding affinity and half life of the mAb or mAbs used, the degree of PSCA expression in the patient, the extent of circulating shed PSCA antigen, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic agents used in combination with the treatment method of the invention. Typical daily doses may range from about 0.1 to 100 mg/kg. Doses in the range of 10-500 mg mAb per week may be effective and well tolerated, although even higher weekly doses may be appropriate and/or well tolerated. The principal determining factor in defining the appropriate dose is the amount of a particular antibody necessary to be therapeutically effective in a particular context. Repeated administrations may be required in order to achieve tumor inhibition or regression. Initial loading doses may be higher. The initial loading dose may be administered as an infusion. Periodic maintenance doses may be administered similarly, provided the initial dose is well tolerated.

Direct administration of PSCA mAbs is also possible and may have advantages in certain contexts. For example, for the treatment of bladder carcinoma, PSCA mAbs may be

injected directly into the bladder. Because PSCA mAbs administered directly to bladder will be cleared from the patient rapidly, it may be possible to use non-human or chimeric antibodies effectively without significant complications of antigenicity.

Patients may be evaluated for serum PSCA in order to assist in the determination of the most effective dosing regimen and related factors. The PSCA Capture ELISA described in Example 20 infra, or a similar assay, may be used for quantitating circulating PSCA levels in patients prior to treatment. Such assays may also be used for monitoring purposes throughout therapy, and may be useful to gauge therapeutic success in combination with evaluating other parameters such as serum PSA levels.

The invention further provides vaccines formulated to contain a PSCA protein or fragment thereof. The use of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity for use in anti-cancer therapy is well known in the art and, for example, has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al., 1995, Int. J. Cancer 63: 231-237; Fong et al., 1997, J. Immunol. 159: 3113-3117). Such methods can be readily practiced by employing a PSCA protein, or fragment thereof, or a PSCA-encoding nucleic acid molecule and recombinant vectors capable of expressing and appropriately presenting the PSCA immunogen.

For example, viral gene delivery systems may be used to deliver a PSCA-encoding nucleic acid molecule. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, 1996, Curr. Opin. Immunol. 8: 658-663). Non-viral delivery systems may also be employed by using naked DNA encoding a PSCA protein or fragment thereof introduced into the patient (e.g., intramuscularly) to induce an anti-tumor response. In one embodiment, the full-length human PSCA cDNA may be employed. In another embodiment, PSCA nucleic acid molecules encoding specific cytotoxic T lymphocyte (CTL) epitopes may be employed. CTL epitopes can be determined using specific

algorithms (e.g., Epimer, Brown University) to identify peptides within a PSCA protein which are capable of optimally binding to specified HLA alleles.

Various ex vivo strategies may also be employed. One approach involves the use of dendritic cells to present PSCA antigen to a patient's immune system. Dendritic cells express MHC class I and II, B7 costimulator, and IL-12, and are thus highly specialized antigen presenting cells. In prostate cancer, autologous dendritic cells pulsed with peptides of the prostate-specific membrane antigen (PSMA) are being used in a Phase I clinical trial to stimulate prostate cancer patients' immune systems (Tjoa et al., 1996, Prostate 28: 65-69; Murphy et al., 1996, Prostate 29: 371-380). Dendritic cells can be used to present PSCA peptides to T cells in the context of MHC class I and II molecules. In one embodiment, autologous dendritic cells are pulsed with PSCA peptides capable of binding to MHC molecules. In another embodiment, dendritic cells are pulsed with the complete PSCA protein. Yet another embodiment involves engineering the overexpression of the PSCA gene in dendritic cells using various implementing vectors known in the art, such as adenovirus (Arthur et al., 1997, Cancer Gene Ther. 4: 17-25), retrovirus (Henderson et al., 1996, Cancer Res. 56: 3763-3770), lentivirus, adeno-associated virus, DNA transfection (Ribas et al., 1997, Cancer Res. 57: 2865-2869), and tumor-derived RNA transfection (Ashley et al., 1997, J. Exp. Med. 186: 1177-1182).

Anti-idiotypic anti-PSCA antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing a PSCA protein. Specifically, the generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic anti-PSCA antibodies that mimic an epitope on a PSCA protein (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J Clin Invest 96: 334-342; Herlyn et al., 1996, Cancer Immunol Immunother 43: 65-76). Such an anti-idiotypic antibody can be used in anti-idiotypic therapy as presently practiced with other anti-idiotypic antibodies directed against tumor antigens.

Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing PSCA.

Using the PSCA-encoding DNA molecules described herein, constructs comprising DNA encoding a PSCA protein/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded PSCA protein/immunogen. The PSCA protein/immunogen may be expressed as a cell surface protein or be secreted. Expression of the PSCA protein/immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against prostate cancer. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used (for review, see information and references published at internet address www.genweb.com).

The invention further provides methods for inhibiting cellular activity (e.g., cell proliferation, activation, or propagation) of a cell expressing multiple PSCA antigens on its cell surface. This method comprises reacting the immunoconjugates of the invention (e.g., a heterogeneous or homogenous mixture) with the cell so that the PSCA antigens on the cell surface forms a complex with the immunoconjugates. The greater the number of PSCA antigens on the cell surface, the greater the number of PSCA-antibody complexes can be used. The greater the number of PSCA-antibody complexes the greater the cellular activity that is inhibited. A subject with a neoplastic or preneoplastic condition can be treated in accordance with this method when the inhibition of cellular activity results in cell death.

A heterogeneous mixture includes PSCA antibodies that recognize different or the same epitope, each antibody being conjugated to the same or different therapeutic agent. A homogenous mixture includes antibodies that recognize the same epitope, each antibody being conjugated to the same therapeutic agent.

The invention further provides methods for inhibiting the biological activity of PSCA by blocking PSCA from binding its ligand. The methods comprises contacting an amount of PSCA with an antibody or immunoconjugate of the invention under conditions that permit a PSCA-immunoconjugate or PSCA-antibody complex thereby blocking PSCA from binding its ligand and inhibiting the activity of PSCA.

In another embodiment, the invention provides methods for selectively inhibiting a cell expressing PSCA antigen by reacting any one or a combination of the immunoconjugates of the invention with the cell in an amount sufficient to inhibit the cell. Such amounts include an amount to kill the cell or an amount sufficient to inhibit cell growth or proliferation. As discussed supra the dose and dosage regimen will depend on the nature of the disease or disorder to be treated associated with PSCA, its population, the site to which the antibodies are to be directed, the characteristics of the particular immunotoxin, and the patient. For example, the amount of immunoconjugate can be in the range of 0.1 to 200 mg/kg of patient weight.

METHODS FOR IDENTIFYING PSCA PROTEINS AND PSCA GENES AND RNA

The invention provides methods for identifying cells, tissues or organisms expressing a PSCA protein or a *PSCA* gene. Such methods can be used to diagnose the presence of cells or an organism that expresses a PSCA protein *in vivo* or *in vitro*. The methods of the present invention are particularly useful in the determining the presence of cells that mediate pathological conditions of the prostate. Specifically, the presence of a PSCA protein can be identified by determining whether a PSCA protein, or nucleic acid encoding a PSCA protein, is expressed. The expression of a PSCA protein can be used as a means for diagnosing the presence of cells, tissues or an organism that expresses a PSCA protein or gene.

A variety of immunological and molecular genetic techniques can be used to determine if a PSCA protein is expressed/produced in a particular cell or sample. In general, an extract containing nucleic acid molecules or an extract containing proteins is prepared. The extract is then assayed to determine whether a PSCA protein, or a PSCA-encoding nucleic acid molecule, is produced in the cell.

Various polynucleotide-based detection methods well known in the art may be employed for the detection of PSCA-encoding nucleic acid molecules and for the detection of PSCA

expressing cells in a biological specimen. For example, RT-PCR methods may be used to selectively amplify a PSCA mRNA or fragment thereof, and such methods may be employed to identify cells expressing PSCA, as described in Example 1 below. In a particular embodiment, RT-PCR is used to detect micrometastatic prostate, bladder or pancreatic cancer cells or circulating prostate, bladder or pancreatic cancer cells. Various other amplification type detection methods, such as, for example, branched DNA methods, and various well known hybridization assays using DNA or RNA probes may also be used for the detection of PSCA-encoding polynucleotides or PSCA expressing cells.

Various methods for the detection of proteins are well known in the art and may be employed for the detection of PSCA proteins and PSCA expressing cells. To perform a diagnostic test based on proteins, a suitable protein sample is obtained and prepared using conventional techniques. Protein samples can be prepared, for example, simply by boiling a sample with SDS. The extracted protein can then be analyzed to determine the presence of a PSCA protein using known methods. For example, the presence of specific sized or charged variants of a protein can be identified using mobility in an electric filed. Alternatively, antibodies can be used for detection purposes. A skilled artisan can readily adapt known protein analytical methods to determine if a sample contains a PSCA protein.

Alternatively, PSCA expression can also be used in methods to identify agents that decrease the level of expression of the PSCA gene. For example, cells or tissues expressing a PSCA protein can be contacted with a test agent to determine the effects of the agent on PSCA expression. Agents that activate PSCA expression can be used as an agonist of PSCA activity whereas agents that decrease PSCA expression can be used as an antagonist of PSCA activity.

PSCA PROMOTER AND OTHER EXPRESSION REGULATORY ELEMENTS

The invention further provides expression control sequences found 5' of the of the newly identified *PSCA* gene in a form that can be used to generate expression vectors and transgenic animals. Specifically, the *PSCA* expression control elements, such as the PSCA

promoter that can readily be identified as being 5' from the ATG start codon in the *PSCA* gene, and can be used to direct the expression of an operably linked protein encoding DNA sequence. Since PSCA expression is predominantly expressed in prostate cells, the expression control elements are particularly useful in directing the expression of an introduced transgene in a tissue specific fashion. A skilled artisan can readily use the PSCA gene promoter and other regulatory elements in expression vectors using methods known in the art.

In eukaryotic cells, the regulatory sequences can be found upstream, downstream and within the coding region of the gene. The eukaryotic regulatory sequences comprise a promoter sequence and sometimes at least one enhancer sequence. In a typical eukaryotic gene, the promoter sequence resides upstream and proximal to the coding region of the gene, and must be oriented in one direction to control expression of the gene. In a typical eukaryotic gene, the enhancer sequences can reside in the upstream, downstream and even within the coding region of the gene, and can be oriented in either direction to enhance or suppress expression of the gene.

The present invention provides a DNA fragment containing 9 kb of sequences upstream of the PSCA coding region. The ability of this PSCA fragment to drive expression of an operatively linked transgene has been tested using a series of chimeric reporter constructs transfected into cells. The chimeric reporter constructs demonstrate an expression pattern similar to that of native endogenous PSCA, and the PSCA fragment drives expression of the transgene when linked in the forward orientation. Thus, this PSCA fragment comprises a PSCA upstream regulatory region that exhibits promoter-like activity.

PSCA transcripts are also present at a significantly higher level in prostate tumor cells but not in benign prostatic hyperplasia. Thus PSCA transcripts are detectable in a prostate-predominant manner, and are detectable at a higher level in prostate tumor samples. The significantly higher level of PSCA transcripts, or over-expression as is known in the art, can be determined by measuring and comparing the amount of detectable PSCA transcripts in a normal prostate with a prostate tumor sample. This comparison can be performed by

methods well known in the art, including Northern analysis and RT-PCR assays, and the differences in transcript levels can be quantitated. Thus, the presence of a measurably different amount of PSCA transcripts (i.e., the number of PSCA transcripts in the test sample exceeds the number from a normal sample) in the samples can be used to indicate the presence of prostate cancer.

PSCA expression is also observed in other human cancers, particularly bladder and pancreatic carcinomas. In the case of bladder carcinoma, the degree of PSCA expression appears to correlate with the severity of the disease, reaching the highest level of overexpression in invasive bladder cancer (See Example 17, below).

The pattern of PSCA transcript and protein accumulation is known, and the PSCA upstream regulatory region has been isolated and characterized. A series of chimeric constructs comprising the PSCA upstream regulatory region operatively linked to a transgene has been tested. The PSCA upstream regulatory region drives expression of the transgene in various prostate cells and cell lines, and in bladder, and to a lesser extent in kidney. Thus, the PSCA upstream region drives expression of a transgene in a prostate-predominant manner.

In preferred embodiments, DNA fragments of 9kb, 6kb, 3kb, and 1kb derived from the 5' upstream region of the PSCA gene, as shown in Figure 42, were produced by techniques described herein. The 9kb PSCA upstream region (pEGFP—PSCA) is involved with gene regulatory activity and was deposited on May 17, 1999 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and has there been identified as follows ATCC No. PTA-80. The 9 kb fragment was obtained by amplification using a T7 primer and RIhPSCA3'-5 (5'-gggaattcgcacagccttcagggtc-3') (SEQ ID NO. 21).

USES OF THE FRAGMENT HAVING GENE REGULATORY ACTIVITY

This invention provides methods (e.g., gene therapy methods) for targeting a gene-of-interest to a cancer cell/site so that the protein encoded by the gene can be expressed thereby directly or indirectly ameliorating the diseased state.

A susceptible cell is introduced with an expression vector that expresses a transgene (e.g., a therapeutic gene) under the control of a PSCA upstream region having significantly increased gene expression activity in tumor cells. The use of an expression vector that expresses a therapeutic gene predominantly in tumor cells will allow expression of the therapeutic genes in target cell, such as prostate, bladder and pancreatic tumor cells.

After infecting a susceptible cell, a transgene (e.g., a therapeutic gene) is driven by a PSCA upstream region having increased gene expression activity in a vector, that expresses the protein encoded by the transgene. The use of a fairly specific prostate specific gene vector will allow selective expression of the specific genes in target cells, e.g., prostate cancer cells.

PSCA regions having increased gene expression activity may be modified, e.g., by sequence mutations, deletions, and insertions, so as to produce derivative molecules. Modifications include multiplying the number of sequences that can bind prostate cell specific regulatory proteins and deleting sequences that are nonfunctional in the PSCA region having gene expression activity. Other modifications include adding enhancers thereby improving the efficiency of the PSCA region having promoter activity. Enhancers may function in a position-independent manner and can be located upstream, within or downstream of the transcribed region.

Derivative molecules would retain the functional property of the PSCA upstream region having increased gene expression activity, namely, the molecule having such substitutions will still permit substantially prostate tissue specific expression of a gene of interest located 3' to the fragment. Modification is permitted so long as the derivative molecules retain its ability to drive gene expression in a substantially prostate specific manner compared to a PSCA fragment having promoter activity alone.

In a preferred embodiment, a vector was constructed by inserting a heterologous sequence (therapeutic gene) downstream of the PSCA upstream region having promoter activity.

Examples of therapeutic genes include suicide genes. These are genes sequences the expression of which produces a protein or agent that inhibits tumor cell growth or tumor cell death (e.g., prostate tumor cells). Suicide genes include genes encoding enzymes (e.g., prodrug enzymes), oncogenes, tumor suppressor genes, genes encoding toxins, genes encoding cytokines, or a gene encoding oncostatin. The purpose of the therapeutic gene is to inhibit the growth of or kill the cancer cell or produce cytokines or other cytotoxic agents which directly or indirectly inhibit the growth of or kill the cancer cells.

Suitable prodrug enzymes include thymidine kinase (TK), xanthine-guanine phosphoribosyltransferase (GPT) gene from E. Coli or E. Coli cytosine deaminase (CD), or hypoxanthine phosphoribosyl transferase (HPRT).

Suitable oncogenes and tumor suppressor genes include neu, EGF, ras (including H, K, and N ras), p53, Retinoblastoma tumor suppressor gene (Rb), Wilm's Tumor Gene Product, Phosphotyrosine Phosphatase (PTPase), and nm23. Suitable toxins include Pseudomonas exotoxin A and S; diphtheria toxin (DT); E. coli LT toxins, Shiga toxin, Shiga-like toxins (SLT-1, -2), ricin, abrin, supporin, and gelonin.

Suitable cytokines include interferons, GM-CSF interleukins, tumor necrosis factor (TNF) (Wong G, et al., Human GM-CSF: Molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. Science 1985; 228:810); WO9323034 (1993); Horisberger MA, et al., Cloning and sequence analyses of cDNAs for interferon- and virus-induced human Mx proteins reveal that they contain putative guanine nucleotide-binding sites: functional study of the corresponding gene promoter. Journal of Virology, 1990 Mar, 64(3):1171-81; Li YP et al., Proinflammatory cytokines tumor necrosis factor-alpha and IL-6, but not IL-1, down-regulate the osteocalcin gene promoter. Journal of Immunology, 1992 Feb 1, 148(3):788-94; Pizarro TT, et al. Induction of TNF alpha and TNF beta gene expression in rat cardiac transplants during allograft rejection.

Transplantation, 1993 Aug, 56(2):399-404). (Breviario F, et al., Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. Journal of Biological Chemistry, 1992 Nov 5, 267(31):22190-7; Espinoza-Delgado I, et al., Regulation of IL-2 receptor subunit genes in human monocytes. Differential effects of IL-2 and IFN-gamma. Journal of Immunology, 1992 Nov 1, 149(9):2961-8; Algate PA, et al., Regulation of the interleukin-3 (IL-3) receptor by IL-3 in the fetal liver-derived FL5.12 cell line. Blood, 1994 May 1, 83(9):2459-68; Cluitmans FH, et al., IL-4 down-regulates IL-2-, IL-3-, and GM-CSF-induced cytokine gene expression in peripheral blood monocytes. Annals of Hematology, 1994 Jun, 68(6):293-8; Lagoo, AS, et al., IL-2, IL-4, and IFN-gamma gene expression versus secretion in superantigen-activated T cells. Distinct requirement for costimulatory signals through adhesion molecules. Journal of Immunology, 1994 Feb 15, 152(4):1641-52; Martinez OM, et al., IL-2 and IL-5 gene expression in response to alloantigen in liver allograft recipients and in vitro. Transplantation, 1993 May, 55(5):1159-66; Pang G, et al., GM-CSF, IL-1 alpha, IL-1 beta, IL-6, IL-8, IL-10, ICAM-1 and VCAM-1 gene expression and cytokine production in human duodenal fibroblasts stimulated with lipopolysaccharide, IL-1 alpha and TNF-alpha. Clinical and Experimental Immunology, 1994 Jun, 96(3):437-43; Ulich TR, et al., Endotoxin-induced cytokine gene expression in vivo. III. IL-6 mRNA and serum protein expression and the in vivo hematologic effects of IL-6. Journal of Immunology, 1991 Apr 1, 146(7):2316-23; Mauviel A, et al., Leukoregulin, a T cell-derived cytokine, induces IL-8 gene expression and secretion in human skin fibroblasts. Demonstration and secretion in human skin fibroblasts. Demonstration of enhanced NF-kappa B binding and NF-kappa B-driven promoter activity. Journal of Immunology, 1992 Nov 1, 149(9):2969-76).

Growth factors include Transforming Growth Factor-α (TGFα) and ß (TGFß), cytokine colony stimulating factors (Shimane M, et al., Molecular cloning and characterization of G-CSF induced gene cDNA. Biochemical and Biophysical Research Communications, 1994 Feb 28, 199(1):26-32; Kay AB, et al., Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. Journal of Experimental Medicine, 1991 Mar 1, 173(3):775-8; de Wit H, et al., Differential regulation

of M-CSF and IL-6 gene expression in monocytic cells. British Journal of Haematology, 1994 Feb, 86(2):259-64; Sprecher E, et al., Detection of IL-1 beta, TNF-alpha, and IL-6 gene transcription by the polymerase chain reaction in keratinocytes, Langerhans cells and peritoneal exudate cells during infection with herpes simplex virus-1. Archives of Virology, 1992, 126(1-4):253-69).

Vectors suitable for use in the methods of the present invention are viral vectors including adenoviruses, lentivirus, retroviral vectors, adeno-associated viral (AAV) vectors.

Preferably, the viral vector selected should meet the following criteria: 1) the vector must be able to infect the tumor cells and thus viral vectors having an appropriate host range must be selected; 2) the transferred gene should be capable of persisting and being expressed in a cell for an extended period of time; and 3) the vector should be safe to the host and cause minimal cell transformation. Retroviral vectors and adenoviruses offer an efficient, useful, and presently the best-characterized means of introducing and expressing foreign genes efficiently in mammalian cells. These vectors have very broad host and cell type ranges, express genes stably and efficiently. The safety of these vectors has been proved by many research groups. In fact many are in clinical trials.

Other virus vectors that may be used for gene transfer into cells for correction of disorders include retroviruses such as Moloney murine leukemia virus (MoMuLV); papovaviruses such as JC, SV40, polyoma; Epstein-Barr Virus (EBV); papilloma viruses, e.g. bovine papilloma virus type I (BPV); vaccinia and poliovirus and other human and animal viruses.

Adenoviruses have several properties that make them attractive as cloning vehicles (Bachettis et al.: Transfer of gene for thymidine kinase-deficient human cells by purified herpes simplex viral DNA. PNAS USA, 1977 74:1590; Berkner, K.L.: Development of adenovirus vectors for expression of heterologous genes. Biotechniques, 1988 6:616; Ghosh-Choudhury G, et al., Human adenovirus cloning vectors based on infectious bacterial plasmids. Gene 1986; 50:161; Hag-Ahmand Y, et al., Development of a helper-independent human adenovirus vector and its use in the transfer of the herpes simplex virus thymidine

kinase gene. J Virol 1986; 57:257; Rosenfeld M, et al., Adenovirus-mediated transfer of a recombinant α_1-antitrypsin gene to the lung epithelium in vivo. Science 1991; 252:431).

For example, adenoviruses possess an intermediate sized genome that replicates in cellular nuclei; many serotypes are clinically innocuous; adenovirus genomes appear to be stable despite insertion of foreign genes; foreign genes appear to be maintained without loss or rearrangement; and adenoviruses can be used as high level transient expression vectors with an expression period up to 4 weeks to several months. Extensive biochemical and genetic studies suggest that it is possible to substitute up to 7-7.5 kb of heterologous sequences for native adenovirus sequences generating viable, conditional, helper-independent vectors (Kaufman R.J.; identification of the component necessary for adenovirus translational control and their utilization in cDNA expression vectors. PNAS USA, 1985 82:689).

AAV is a small human parvovirus with a single stranded DNA genome of approximately 5 kb. This virus can be propagated as an integrated provirus in several human cell types. AAV vectors have several advantage for human gene therapy. For example, they are trophic for human cells but can also infect other mammalian cells; (2) no disease has been associated with AAV in humans or other animals; (3) integrated AAV genomes appear stable in their host cells; (4) there is no evidence that integration of AAV alters expression of host genes or promoters or promotes their rearrangement; (5) introduce genes can be rescued from the host cell by infection with a helper virus such as adenovirus.

HSV-1 vector system facilitates introduction of virtually any gene into non-mitotic cells (Geller et al. an efficient deletion mutant packaging system for a defective herpes simplex virus vectors: Potential applications to human gene therapy and neuronal physiology. PNAS USA, 1990 87:8950).

Another vector for mammalian gene transfer is the bovine papilloma virus-based vector (Sarver N, et al., Bovine papilloma virus DNA: A novel eukaryotic cloning vector. Mol Cell Biol 1981; 1:486).

Vaccinia and other poxvirus-based vectors provide a mammalian gene transfer system. Vaccinia virus is a large double-stranded DNA virus of 120 kilodaltons (kd) genomic size (Panicali D, et al., Construction of poxvirus as cloning vectors: Insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccine virus. Proc Natl Acad Sci USA 1982; 79:4927; Smith et al. infectious vaccinia virus recombinants that express hepatitis B virus surface antigens. Nature, 1983 302:490.)

Retroviruses are packages designed to insert viral genes into host cells (Guild B, et al., Development of retrovirus vectors useful for expressing genes in cultured murine embryonic cells and hematopoietic cells in vivo. J Virol 1988; 62:795; Hock RA, et al., Retrovirus mediated transfer and expression of drug resistance genes in human hemopoietic progenitor cells. Nature 1986; 320:275).

The basic retrovirus consists of two identical strands of RNA packaged in a proviral protein. The core surrounded by a protective coat called the envelope, which is derived from the membrane of the previous host but modified with glycoproteins contributed by the virus.

Preferably, for treating defects, disease or damage of cells in, for example, the prostate, vectors of the invention include a therapeutic gene or transgenes, for example a gene encoding TK. The genetically modified vectors are administered into the prostate to treat defects, disease such as prostate cancer by introducing a therapeutic gene product or products into the prostate that enhance the production of endogenous molecules that have ameliorative effects in vivo. The same principles apply with respect to the treatment of other cancers, such as pancreatic, bladder or other cancers expressing PSCA.

The basic tasks in this embodiment of the present method of the invention are isolating the gene of interest, attaching it to a fragment having gene regulatory activity, selecting the proper vector vehicle to deliver the gene of interest to the body, administering the vector having the gene of interest into the body, and achieving appropriate expression of the gene of interest to a target cell. The present invention provides packaging the cloned genes, i.e. the genes of interest, in such a way that they can be injected directly into the bloodstream or

relevant organs of patients who need them. The packaging will protect the foreign DNA from elimination by the immune system and direct it to appropriate tissues or cells.

Along with the human or animal gene of interest another gene, e.g., a selectable marker, can be inserted that will allow easy identification of cells that have incorporated the modified retrovirus. The critical focus on the process of gene therapy is that the new gene must be expressed in target cells at an appropriate level with a satisfactory duration of expression.

The methods described below to modify vectors and administering such modified vectors into the target organ (e.g., prostate) are merely for purposes of illustration and are typical of those that might be used. However, other procedures may also be employed, as is understood in the art.

Most of the techniques used to construct vectors and the like are widely practiced in the art, and most practitioners are familiar with the standard resource materials which describe specific conditions and procedures. However, for convenience, the following paragraphs may serve as a guideline.

GENERAL METHODS FOR VECTOR CONSTRUCTION

Construction of suitable vectors containing the desired therapeutic gene coding and control sequences employs standard ligation and restriction techniques, which are well understood in the art (see Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1982)). Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and religated in the form desired.

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes (See, e.g. New England Biolabs Product Catalog). In general, about 1 μg of plasmid or DNA sequences is cleaved by one unit of enzyme in about 20 μl of buffer solution.

Typically, an excess of restriction enzyme is used to insure complete digestion of the DNA substrate.

Incubation times of about one hour to two hours at about 37°C are workable, although variations can be tolerated. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in Methods in Enzymology 65:499-560 (1980).

Restriction cleaved fragments may be blunt ended by treating with the large fragment of E. coli DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using incubation times of about 15 to 25 min at 20°C to 25°C in 50 mM Tris (pH 7.6) 50 mM NaCl, 6 mM $MgCl_2$, 6 mM DTT and 5-10 μM dNTPs. The Klenow fragment fills in at 5' sticky ends but chews back protruding 3' single strands, even though the four dNTPs are present. If desired, selective repair can be performed by supplying only one of the dNTPs, or with selected dNTPs, within the limitations dictated by the nature of the sticky ends. After treatment with Klenow, the mixture is extracted with phenol/chloroform and ethanol precipitated. Treatment under appropriate conditions with Sl nuclease or Bal-31 results in hydrolysis of any single-stranded portion.

Ligations are performed in 10-50 μl volumes under the following standard conditions and temperatures using T4 DNA ligase. Ligation protocols are standard (D. Goeddel (ed.) Gene Expression Technology: Methods in Enzymology (1991)).

In vector construction employing "vector fragments", the vector fragment is commonly treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase (CIP) in order to remove the 5' phosphate and prevent religation of the vector. Alternatively, religation can be prevented in vectors which have been double digested by additional restriction enzyme digestion of the unwanted fragments.

Suitable vectors include viral vector systems e.g. ADV, RV, and AAV (R.J. Kaufman "Vectors used for expression in mammalian cells" in Gene Expression Technology, edited by D.V. Goeddel (1991).

Many methods for inserting functional DNA transgenes into cells are known in the art. For example, non-vector methods include nonviral physical transfection of DNA into cells; for example, microinjection (DePamphilis et al., BioTechnique 6:662-680 (1988)); liposomal mediated transfection (Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987), Felgner and Holm, Focus 11:21-25 (1989) and Felgner et al., Proc. West. Pharmacol. Soc. 32: 115-121 (1989)) and other methods known in the art.

ADMINISTRATION OF MODIFIED VECTORS INTO SUBJECT

One way to get DNA into a target cell is to put it inside a membrane bound sac or vesicle such as a spheroplast or liposome, or by calcium phosphate precipitation ($CaPO_4$) (Graham F. and Van der Eb, A., Virology 52:456 1973; Schaefer-Ridder M., et al., Liposomes as gene carriers: Efficient transduction of mouse L cells by thymidine kinase gene. Science 1982; 215:166; Stavridis JC, et al., Construction of transferrin-coated liposomes for in vivo transport of exogenous DNA to bone marrow erythroblasts in rabbits. Exp Cell Res 1986; 164:568-572).

A vesicle can be constructed in such a way that its membrane will fuse with the outer membrane of a target cell. The vector of the invention in vesicles can home into the target cells. The spheroplasts are maintained in high ionic strength buffer until they can be fused through the mammalian target cell using fusogens such as polyethylene glycol.

Liposomes are artificial phospholipid vesicles. Vesicles range in size from 0.2 to 4.0 micrometers and can entrap 10% to 40% of an aqueous buffer containing macromolecules. The liposomes protect the DNA from nucleases and facilitate its introduction into target cells. Transfection can also occur through electroporation.

Before administration, the modified vectors are suspended in complete PBS at a selected density for injection. In addition to PBS, any osmotically balanced solution which is physiologically compatible with the subject may be used to suspend and inject the modified vectors into the host.

For injection, the cell suspension is drawn up into the syringe and administered to anesthetized recipients. Multiple injections may be made using this procedure. The viral suspension procedure thus permits administration of genetically modified vectors to any predetermined site in the target organ (e.g., prostate), is relatively non-traumatic, allows multiple administrations simultaneously in several different sites or the same site using the same viral suspension. Multiple injections may consist of a mixture of therapeutic genes.

USES OF THE MODIFIED VECTORS

The present invention provides methods for maintaining and increasing expression of therapeutic genes using a fragment having expression activity.

The methods of the invention are exemplified by embodiments in which modified vectors carrying a therapeutic gene are injected into a subject.

In a first embodiment a protein product is expressed comprising growing the host vector system of the invention so as to produce the protein in the host and recovering the protein so produced. This method permits the expression of genes of interest in both unicellular and multicellular organisms. For example, in an in vitro assay, prostate cells having the vector of the invention comprising a gene of interest (e.g., the ras gene) may be used in microtiter wells as an unlimited for the ras gene product. A sample from a subject would be added to the wells to detect the presence of antibodies directed against the ras gene. This assay can aid in the quantitative and qualitative determination of the presence of ras antibodies in the sample for the clinical assessment of whether the subject's immune system is combatting the disease associated with elevated levels of ras.

In a second embodiment metastatic prostate cancer is treated via gene therapy, i.e., the correction of a disease phenotype in vivo through the use of the nucleic acid molecules of the invention.

In accordance with the practice of this invention, the subject of the gene therapy may be a human, equine, porcine, bovine, murine, canine, feline, or avian subject. Other mammals are also included in this invention.

The most effective mode of administration and dosage regimen for the molecules of the present invention depends upon the exact location of the prostate tumor being treated, the severity and course of the cancer, the subject's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the molecules should be titrated to the individual subject. The molecules may be delivered directly or indirectly via another cell, autologous cells are preferred, but heterologous cells are encompassed within the scope of the invention.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m^2 of surface area is described by Freireich, E.J., et al. Cancer Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen may be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses may be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided dose may be administered daily or proportionally reduced depending on the specific therapeutic situation).

It would be clear that the dose of the molecules of the invention required to achieve treatment may be further modified with schedule optimization.

GENERATION OF TRANSGENIC ANIMALS

Another aspect of the invention provides transgenic non-human mammals comprising PSCA nucleic acids. For example, in one application, PSCA-deficient non-human animals can be generated using standard knock-out procedures to inactivate a *PSCA* homologue or, if such animals are non-viable, inducible *PSCA* homologue antisense molecules can be used to regulate PSCA homologue activity/expression. Alternatively, an animal can be altered so as to contain a human PSCA-encoding nucleic acid molecule or an antisense-PSCA expression unit that directs the expression of PSCA protein or the antisense molecule in a tissue specific fashion. In such uses, a non-human mammal, for example a mouse or a rat, is generated in which the expression of the *PSCA* homologue gene is altered by inactivation or activation and/or replaced by a human *PSCA* gene. This can be accomplished using a variety of art-known procedures such as targeted recombination. Once generated, the PSCA homologue deficient animal, the animal that expresses PSCA (human or homologue) in a tissue specific manner, or an animal that expresses an antisense molecule can be used to (1) identify biological and pathological processes mediated by the PSCA protein, (2) identify proteins and other genes that interact with the PSCA proteins, (3) identify agents that can be exogenously supplied to overcome a PSCA protein deficiency and (4) serve as an appropriate screen for identifying mutations within the *PSCA* gene that increase or decrease activity.

For example, it is possible to generate transgenic mice expressing the human minigene encoding PSCA in a tissue specific-fashion and test the effect of over-expression of the protein in tissues and cells that normally do not contain the PSCA protein. This strategy has been successfully used for other genes, namely bcl-2 (Veis et al. Cell 1993 75:229). Such an approach can readily be applied to the PSCA protein/gene and can be used to address the issue of a potential beneficial or detrimental effect of the PSCA proteins in a specific tissue.

Further, in another embodiment, the invention provides a transgenic animal having germ and somatic cells comprising an oncogene which is linked to a PSCA upstream region effective for the expression of said gene in the tissues of said mouse for the promotion of a cancer associated with the oncogene, thereby producing a mouse model of that cancer.

COMPOSITIONS

The invention provides a pharmaceutical composition comprising a PSCA nucleic acid molecule of the invention or an expression vector encoding a PSCA protein or encoding a fragment thereof and, optionally, a suitable carrier. The invention additionally provides a pharmaceutical composition comprising an antibody or fragment thereof which recognizes and binds a PSCA protein. In one embodiment, the antibody or fragment thereof is conjugated or linked to a therapeutic drug or a cytotoxic agent.

Suitable carriers for pharmaceutical compositions include any material which when combined with the nucleic acid or other molecule of the invention retains the molecule's activity and is non-reactive with the subject's immune systems. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including coated tablets and capsules. Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods. Such compositions may also be formulated within various lipid compositions, such as, for example, liposomes as well as in various polymeric compositions, such as polymer microspheres.

The invention also provides a diagnostic composition comprising a PSCA nucleic acid molecule, a probe that specifically hybridizes to a nucleic acid molecule of the invention or to any part thereof, or a PSCA antibody or fragment thereof. The nucleic acid molecule, the probe or the antibody or fragment thereof can be labeled with a detectable marker. Examples of a detectable marker include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator or an enzyme. Further, the invention provides a diagnostic composition

comprising a PSCA-specific primer pair capable of amplifying PSCA-encoding sequences using polymerase chain reaction methodologies, such as RT-PCR.

EXAMPLES

Example 1 :

Identification And Molecular Characterization Of A Novel Prostate Cell Surface Antigen (PSCA)

MATERIALS AND METHODS

LAPC-4 Xenografts: LAPC-4 xenografts were generated as described in Klein et al, 1997, Nature Med. 3: 402-408.

RDA, Northern Analysis and RT-PCR: Representational difference analysis of androgen dependent and independent LAPC-4 tumors was performed as previously described (Braun et al., 1995, Mol. Cell. Biol. 15: 4623-4630). Total RNA was isolated using Ultraspec^R RNA isolation systems (Biotecx, Houston, TX) according to the manufacturer's instructions. Northern filters were probed with a 660bp RDA fragment corresponding to the coding sequence and part of the 3' untranslated sequence of PSCA or a ~400bp fragment of PSA. The human multiple tissue blot was obtained from Clontech and probed as specified. For reverse transcriptase (RT)-PCR analysis, first strand cDNA was synthesized from total RNA using the GeneAmp RNA PCR core kit (Perkin Elmer-Roche, New Jersey). For RT-PCR of human PSCA transcripts, primers 5'-tgcttgccctgttgatggcag- (SEQ ID NO. 19) and 3'- ccagagcagcaggccgagtgca- (SEQ ID NO. 20) were used to amplify a ~320 bp fragment. Thermal cycling was performed by 25-25 cycles of 95° for 30 sec, 60° for 30sec and 72° for 1 min, followed by extension at 72° for 10 min. Primers for GAPDH (Clontech) were used as controls. For mouse PSCA, the primers used were 5' -ttctcctgctggccacctac- and 3' -gcagctcatcccttcacaat-.

In Situ Hybridization Assay for PSCA mRNA: For mRNA in situ hybridization, recombinant plasmid pCR II (1 ug, Invitrogen, San Diego, CA) containing the full-length PSCA gene was linearized to generate sense and antisense digoxigenin labeled riboprobes. In situ hybridization was performed on an automated instrument (Ventana Gen II, Ventana Medical Systems) as previously described (Magi-Galluzzi et al., 1997, Lab. Invest. 76: 37-43). Prostate specimens were obtained from a previously described database which has been expanded to ~130 specimens (Magi-Galluzzi et al., supra). Slides were read and scored by two pathologists in a blinded fashion. Scores of 0-3 were assigned according to the percentage of positive cells (0 = 0%; 1 = <25%; 2 = 25-50%; 3 = >50%) and the intensity of staining (0 = 0; 1 = 1+; 2 = 2+; 3 = 3+). The two scores were multiplied to give an overall score of 0-9.

RESULTS

Human PSCA cDNA: Representational Difference Analysis (RDA), a PCR-based subtractive hybridization technique, was used to compare gene expression between hormone dependent and hormone independent variants of a human prostate cancer xenograft (LAPC-4) and to isolate cDNAs upregulated in the androgen-independent LAPC-4 subline. Multiple genes were cloned, sequenced, and checked for differential expression. One 660bp fragment (clone #15) was identified which was found to be highly overexpressed in xenograft tumors when compared with normal prostate. Comparison of the expression of this clone to that of PSA in normal prostate and xenograft tumors suggested that clone #15 was relatively cancer specific (FIG. 9).

Sequence analysis revealed that clone #15 had no exact match in the databases, but shared 30% nucleotide homology with stem cell antigen 2, a member of the Thy-1/Ly-6 superfamily of glycosylphosphatidylinositol (GPI)-anchored cell surface antigens. Clone #15 encodes a 123 amino acid protein (SEQ ID NO:2) which is 30% identical to SCA-2 (SEQ ID NO:5) (also called RIG-E) and contains a number of highly conserved cysteine residues characteristic of the Ly-6/Thy-1 gene family (FIG. 3). Consistent with its homology to a family of GPI-anchored proteins, clone #15 contains both an amino-

terminal hydrophobic signal sequence and a carboxyl-terminal stretch of hydrophobic amino acids preceded by a group of small amino acids defining a cleavage/binding site for GPI linkage (Udenfriend and Kodukula, 1995, Ann. Rev. Biochem. 64: 563-591). It also contains four predicted N-glycosylation sites. Because of its strong homology to the stem cell antigen-2, clone #15 was renamed prostate stem cell antigen (PSCA). 5' and 3' PCR RACE analysis was then performed using cDNA obtained from the LAPC-4 androgen independent xenograft and the full length cDNA nucleotide sequence (including the coding and untranslated regions) was obtained. The nucleotide sequence of the full length cDNA (SEQ ID NO:1) encoding human PSCA is shown in FIG. 1A and the translated amino acid sequence (SEQ ID NO:2) is shown in FIG. 1B and in FIG. 3.

PSCA is expressed in prostate cells: The distribution of PSCA mRNA in normal human tissues was examined by Northern blot analysis. The results, shown in FIG. 9B, demonstrate that PSCA is expressed predominantly in prostate, with a lower level of expression present in placenta. Small amounts of mRNA can be detected in kidney and small intestine after prolonged exposure and at approximately 1/100th of the level seen in prostate tissue. RT-PCR analysis of PSCA expression in normal human tissues also demonstrates that PSCA expression is restricted. In a panel of normal tissues, high level PSCA mRNA expression was detected in prostate, with significant expression detected in placenta and tonsils (FIG. 7A). RT-PCR analysis of PSCA mRNA expression in a variety of prostate cancer xenografts prostate cancer cell lines and other cell lines, and normal prostate showed high level expression restricted to normal prostate, the LAPC-4 and LAPC-9 prostate cancer xenografts, and the ovarian cancer cell line A431 (FIG. 7B). The major PSCA transcript in normal prostate is ~1kb (FIG. 9B). Mouse PSCA expression was analyzed by RT-PCR in mouse spleen, liver, lung, prostate, kidney and testis. Like human PSCA, murine PSCA is expressed predominantly in prostate. Expression can also be detected in kidney at a level similar to that seen for placenta in human tissues.

The expression of PSCA, PSMA and PSA in prostate cancer cell lines and xenografts was compared by Northern blot analysis. The results shown in FIG. 10 demonstrate high

level prostate cancer specific expression of both PSCA and PSMA, whereas PSA expression is not prostate cancer specific.

PSCA is Expressed by a Subset of Basal Cells in Normal Prostate: Normal prostate contains two major epithelial cell populations--secretory luminal cells and subjacent basal cells. In situ hybridizations were performed on multiple sections of normal prostate using an antisense riboprobe specific for PSCA to localize its expression. As shown in FIG. 11, PSCA is expressed exclusively in a subset of normal basal cells. Little to no staining is seen in stroma, secretory cells or infiltrating lymphocytes. Hybridization with sense PSCA riboprobes showed no background staining. Hybridization with an antisense probe for GAPDH confirmed that the RNA in all cell types was intact. Because basal cells represent the putative progenitor cells for the terminally differentiated secretory cells, these results suggest that PSCA may be a prostate-specific stem/progenitor cell marker (Bonkhoff et al., 1994, Prostate 24: 114-118). In addition, since basal cells are androgen-independent, the association of PSCA with basal cells raises the possibility that PSCA may play a role in androgen-independent prostate cancer progression.

PSCA is Overexpressed in Prostate Cancer Cells: The initial analysis comparing PSCA expression in normal prostate and LAPC-4 xenograft tumors suggested that PSCA was overexpressed in prostate cancer. As demonstrated by the Northern blot analysis as shown in FIG. 9, LAPC-4 prostate cancer tumors strongly express PSCA; however, there is almost no detectable expression in sample of BPH. In contrast, PSA expression is clearly detectable in normal prostate, at levels 2-3 times those seen in the LAPC-4 tumors. Thus, the expression of PSCA in prostate cancer appears to be the reverse of what is seen with PSA. While PSA is expressed more strongly in normal than malignant prostate tissue, PSCA is expressed more highly in prostate cancer.

To confirm the PSCA expression and its value in diagnosing prostate cancer, one hundred twenty six paraffin-embedded prostate cancer specimens were analyzed by mRNA in situ hybridization for PSCA expression. Specimens were obtained from primary tumors removed by radical prostatectomy or transurethral resection in all cases except one. All

specimens were probed with both a sense and antisense construct in order to control for background staining. Slides were assigned a composite score as describe under Materials and Methods, with a score of 6 to 9 indicating strong expression and a score of 4 meaning moderate expression. 102/126 (81%) of cancers stained strongly for PSCA, while another 9/126 (7%) displayed moderate staining (FIGS. 11B and 11C). High grade prostatic intraepithelial neoplasia, the putative precursor lesion of invasive prostate cancer, stained strongly positive for PSCA in 82% (97/118) of specimens (FIG. 11B) (Yang et al., 1997, Am. J. Path. 150: 693-703). Normal glands stained consistently weaker than malignant glands (FIG. 11B). Nine specimens were obtained from patients treated prior to surgery with hormone ablation therapy. Seven of nine (78%) of these residual presumably androgen-independent cancers overexpressed PSCA, a percentage similar to that seen in untreated cancers. Because such a large percentage of specimens expressed PSCA mRNA, no statistical correlation could be made between PSCA expression and pathological features such as tumor stage and grade. These results suggest that PSCA mRNA overexpression is a common feature of androgen-dependent and independent prostate cancer.

PSCA is Expressed in Androgen Independent Prostate Cancer Cell Lines: Although PSCA was initially cloned using subtractive hybridization, Northern blot analysis demonstrated strong PSCA expression in both androgen-dependent and androgen-independent LAPC-4 xenograft tumors (FIG. 9). Moreover, PSCA expression was detected in all prostate cancer xenografts, including the LAPC-4 and LAPC-9 xenografts.

PSCA expression in the androgen-independent, androgen receptor-negative prostate cancer cell lines PC3 and DU145 was also detected by reverse-transcriptase polymerase chain reaction analysis. These data suggest that PSCA can be expressed in the absence of functional androgen receptor.

Example 2:

Biochemical Characterization Of PSCA

This experiment shows that PSCA is a glycosylated, GPI-anchored cell surface protein.

MATERIALS AND METHODS

Polyclonal Antibodies and Immunoprecipitations: Rabbit polyclonal antiserum was generated against the synthetic peptide -TARIRAVGLLTVISK- (SEQ ID NO. 16) and affinity purified using a PSCA-glutathione S transferase fusion protein. 293T cells were transiently transfected with pCDNA II (Invitrogen, San Diego, CA) expression vectors containing PSCA, CD59, E25 or vector alone by calcium phosphate precipitation. Immunoprecipitation was performed as previously described (Harlow and Lane, 1988, Antibodies: A Laboratory Manual. (Cold Spring Harbor Press)). Briefly, cells were labeled with 500uCi of trans35S label (ICN, Irvine, CA) for six hours. Cell lysates and conditioned media were incubated with 1ug of purified rabbit anti-PSCA antibody and 20ul protein A sepharose CL-4B (Pharmacia Biotech, Sweden) for two hours. For deglycosylation, immunoprecipitates were treated overnight at 37° with 1u N-glycosidase F (Boehringer Mannheim) or 0.1 u neuraminidase (Sigma, St. Louis, MO) for 1 hour followed by overnight in 2.5 mU O-glycosidase (Boehringer Mannheim).

Flow Cytometry: For flow cytometric analysis of PSCA cell surface expression, single cell suspensions were stained with 2ug/ml of purified anti-PSCA antibody and a 1:500 dilution of fluorescein isothiocyanate (FITC) labeled anti-rabbit IgG (Jackson Laboratories, West Grove, PA). Data was acquired on a FACScan (Becton Dickinson) and analyzed using LYSIS II software. Control samples were stained with secondary antibody alone. Glycosylphosphatidyl inositol linkage was analyzed by digestion of 2 X 10^6 cells with 0.5 units of phosphatidylinositol-specific phospholipase C (PI-PLC, Boehringer Mannheim) for 90 min at 37° C. Cells were analyzed prior to and after digestion by either FACS scanning or immunoblotting.

RESULTS

PSCA is a GPI-Anchored Glycoprotein Expressed on the Cell Surface: The deduced PSCA amino acid sequence predicts that PSCA is heavily glycosylated and anchored to the cell surface through a GPI mechanism. In order to test these predictions, we produced an affinity purified polyclonal antibody raised against a unique PSCA peptide (see Materials and Methods). This peptide contains no glycosylation sites and was predicted, based on comparison to the three dimensional structure of CD59 (another GPI-anchored PSCA homologue), to lie in an exposed portion of the mature protein (Kiefer et al., 1994, Biochem. 33: 4471-4482). Recognition of PSCA by the affinity-purified antibody was demonstrated by immunoblot and immunoprecipitation analysis of extracts of 293T cells transfected with PSCA and a GST-PSCA fusion protein. The polyclonal antibody immunoprecipitates predominantly a 24kd band from PSCA-transfected, but not mock-transfected cells (FIG. 12A). Three smaller bands are also present, the smallest being ~10kd. The immunoprecipitate was treated with N and O specific glycosidases in order to determine if these bands represented glycosylated forms of PSCA. N-glycosidase F deglycosylated PSCA, whereas O-glycosidase had no effect (FIG. 12A). Some GPI-anchored proteins are known to have both membrane-bound and secreted forms (Fritz and Lowe, 1996, Am. J. Physiol. 270: G176-G183). FIG. 12B indicates that some PSCA is secreted in the 293T-overexpressing system. The secreted form of PSCA migrates at a lower molecular weight than the cell surface-associated form, perhaps reflecting the absence of the covalent GPI-linkage. This result may reflect the high level of expression in the 293T cell line and needs to be confirmed in prostate cancer cell lines and *in vivo*.

Fluorescence activated cell sorting (FACS) analysis was used to localize PSCA expression to the cell surface. Nonpermeabilized mock-transfected 293T cells, PSCA-expressing 293T cells and LAPC-4 cells were stained with affinity purified antibody or secondary antibody alone. FIG. 12C shows cell surface expression of PSCA in PSCA-transfected 293T and LAPC-4 cells, but not in mock-transfected cells. To confirm that this cell surface expression is mediated by a covalent GPI-linkage, cells were treated with GPI-specific phospholipase C (PLC). Release of PSCA from the cell surface by PLC was indicated by a greater than one log reduction in fluorescence intensity. Recovery of PSCA in post digest conditioned medium was also confirmed by immunoblotting. The

specificity of phospholipase C digestion for GPI-anchored proteins was confirmed by performing the same experiment on 293T cells transfected with the GPI-linked antigen CD59 or the non-GPI linked transmembrane protein E25a (Deleersnijder et al., 1996, J. Biol. Chem 271: 19475-19482). PLC digestion reduced cell surface expression of CD59 to the same degree as PSCA but had no effect on E25. These results support the prediction that PSCA is a glycosylated, GPI-anchored cell surface protein.

Example 3:

Isolation Of cDNA Encoding Murine PSCA Homologue

The human PSCA cDNA was used to search murine EST databases in order to identify homologues for potential transgenic and knockout experiments. One EST obtained from fetal mouse and another from neonatal kidney were 70% identical to the human cDNA at both the nucleotide and amino acid levels. The homology between the mouse clones and human PSCA included regions of divergence between human PSCA and its GPI-anchored homologues, indicating that these clones likely represented the mouse homologue of PSCA. Alignment of these ESTs and 5' extension using RACE-PCR provided the entire coding sequence (SEQ ID NO:4) (FIG. 2).

Example 4:

Isolation Of Human And Murine PSCA Genes

This experiment shows that PSCA is located at chromosome 8, band q24.2.

MATERIALS AND METHODS

Genomic Cloning: Lambda phage clones containing the human PSCA gene were obtained by screening a human genomic library (Stratagene) with a human PSCA cDNA probe (Sambrook et al., 1989, Molecular Cloning (Cold Spring Harbor)). BAC (bacterial

artificial chromosome) clones containing the murine PSCA gene were obtained by screening a murine BAC library (Genome Systems, Inc., St. Louis, MO) with a murine PSCA cDNA probe. A 14kb human Not I fragment and a 10kb murine Eco RI fragment were subcloned into pBluescript (Stratagene), sequenced, and restriction mapped.

Chromosome Mapping by Fluorescence In Situ Hybridization: Fluorescence in situ chromosomal analysis (FISH) was performed as previously described using overlapping human lambda phage clones (Rowley et al., 1990, PNAS USA 87: 9358-9362, H. Shizuya, PNAS USA, 89:8794).

RESULTS

Structure of PSCA Gene: Human and murine genomic clones of approximately 14kb and 10kb, respectively, were obtained and restriction mapped. A schematic representation of the gene structures of human and murine PSCA and Ly-6/Thy-1 is shown in FIG. 8. Both the human and murine genomic clones contain three exons encoding the translated and 3' untranslated regions of the PSCA gene. A fourth exon encoding a 5' untranslated region is presumed to exist based on PSCA's homology to other members of the Ly-6 and Thy-1 gene families (FIG. 8).

Human PSCA Gene Maps to Chromosome 8q24.2: Southern blot analysis of LAPC-4 genomic DNA revealed that PSCA is encoded by a single copy gene. Other Ly-6 gene family members contain four exons, including a first exon encoding a 5' untranslated region and three additional exons encoding the translated and 3' untranslated regions. Genomic clones of human and murine PSCA containing all but the presumed 5' first exon were obtained by screening lambda phage libraries. Mouse and human PSCA clones had a similar genomic organization. The human clone was used to localize PSCA by fluorescence in situ hybridization analysis. Cohybridization of overlapping human PSCA lambda phage clones resulted in specific labeling only of chromosome 8 (FIG. 13). Ninety seven percent of detected signals localized to chromosome 8q24, of which 87%

were specific for chromosome 8q24.2. These results show that PSCA is located at chromosome 8, band q24.2.

Example 5:

Generation Of Monoclonal Antibodies Recognizing Different Epitopes Of PSCA

MATERIALS AND METHODS

Generation and Production of Monoclonal Antibodies: BALB/c mice were immunized three times with a purified PSCA-glutathione S-transferase (GST) fusion protein containing PSCA amino acids 22-99 (SEQ ID NO:2) (FIG. 1B). Briefly, the PSCA coding sequence corresponding to amino acids 18 through 98 of the human PSCA amino acid sequence was PCR-amplified using the primer pair:

5'- GGAGAATTCATGGCACTGCCCTGCTGTGCTAC (SEQ ID NO. 22)

3'-GGAGAATTCCTAATGGGCCCCGCTGGCGTT (SEQ ID NO. 23)

The amplified PSCA sequence was cloned into pGEX-2T (Pharmacia), used to transform E. coli, and the fusion protein isolated.

Spleen cells were fused with HL-1 myeloma cells using standard hybridoma technique. Hybridomas that were positive for PSCA by ELISA and FACS analysis (see Results) were subcloned. Ascites fluid was produced in C.B. 17 scid/scid mice and monoclonal antibodies (mAbs) purified using a protein G affinity column (Pharmacia Biotech, Piscataway, N.J.). PSCA mAb 1G8 was also produced in Cell-Pharm System 100 as recommended by the manufacturer (Unisyn Technologies, Hopkinton, MA).

ELISA for Hybridoma Screening: GST or PSCA-GST were immobilized on Reacti-Bind maleic anhydride-activated polystyrene plates (Pierce, Rockford, IL). 50ul of hybridoma media were added to each well and incubated for 1 hour at room temperature. Wells were

washed 3 times with 200ul PBS containing 0.1% BSA and 0.05% Tween 20 and incubated for 1 hour with 100ul anti-mouse IgG (1:4000) labeled with alkaline phosphatase (Promega, Madison, WI). Plates were developed with an alkaline phosphatase substrate (Bio-Rad, Hercules, CA).

Cell Culture: LNCaP was obtained from ATCC and stably transfected with a pCDNA II (Invitrogen) expression vector containing PSCA or vector alone (Reiter, R. et al., 1998). 293T cells transiently transfected with PSCA or vector alone were prepared as described previously (Reiter, R. et al., 1998). LAPC-9 xenograft explants were propagated in PrEGM media (Clonetics, San Diego, CA) after digestion in 1% pronase for 18 min. at room temperature. Before FACS analysis, LAPC-9 cells were passed though a 40um cell strainer to obtain single cell suspensions.

Immunofluorescence: Cells were grown on glass coverslips coated with poly-L-lysine. Immunofluorescence assays were carried out on permeabilized and nonpermeabilized fixed cells. For fixation, cells were treated with 2% paraformaldehyde in PBS-CM (PBS, 100uM $CaCl_2$, 1mM $MgCl_2$) for 30 minutes in the dark, quenched with 50uM NH_4Cl in PBS-CM-BSA (PBS, 100uM $CaCl_2$, 1mM $MgCl_2$, 0.2% BSA) for 10 minutes, and washed twice with PBS-CM-BSA. For permeabilization, cells were treated additionally with PBS-CM-BSA-Saponin (0.075% saponin (Sigma) in PBS-CM-BSA) for 15 minutes at room temperature. Primary mAb at 2-5 mg/ml in PBS-CM-BSA (plus saponin in cases of permeabilization) was added for 60 minutes and washed twice with PBS-CM-BSA. FITC-conjugated goat antimouse IgG antibody (1:500 diluted in PBS-CM-BSA +/- saponin; Southern Biotechnology, Birmingham, AL) was added for 30 minutes and washed 3 times with PBS-CM. Slides were mounted in vectashield (Vector Laboratory, Inc., Burlingame, CA).

Flow Cytometry: Cells (1 X 10^6) were incubated for 30 minutes at 4° C with 100 ul mAb at 2ug/ml in PBS containing 2% fetal bovine serum or hybridoma conditioned medium. After washing, cells were stained with a 1:500 dilution of FITC-conjugated goat

antimouse IgG (Southern Biotechnology, Birmingham, AL). Data was acquired on a FACScan (Becton Dickinson) and analyzed by using LYSIS II software.

Immunoblotting and Immunoprecipitation: Immunoprecipitation was performed as described (Harlow, E. and Lane, D., 1988). Briefly, cells were labeled with 500uCi of trans35 label (ICN) for 6 hours. Cell lysates were incubated with 3ug mAb and 20ul of protein A-Sepharose CL-4B (Pharmacia Biotech) for 2 hours. For immunoblotting, protein extracts were prepared by lysing cells in 1X SDS Laemmli sample buffer and boiling for 5 min. Proteins were separated on 12.5% SDS polyacrylamide gels and transferred to nitrocellulose membranes, washed and incubated with 2ug mAb in 10ml blocking buffer (5% nonfat milk in TBST). Blots were developed using the Amersham enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL).

Immunohistochemistry: Normal formalin-fixed, paraffin-embedded tissue samples were obtained from the Departments of Pathology at Beth-Israel Deaconess Medical Center-Harvard Medical School and UCLA. Primary radical prostatectomy specimens were selected from a previously described database (Magi-Galluzzi, C.et al., 1997). Bone metastases and matched primary biopsy specimens were obtained from the UCLA Department of Pathology. Normal tissues were stained and scored independently at two institutions in order to ensure reproducibility. Specimens obtained from UCLA were stained using modifications of an immunoperoxidase technique previously described (Said, J. W. et al., 1998). Antigen retrieval was performed on paraffin sections using a commercial steamer and 0.01M citrate buffer pH 6.0. After incubation with PSCA mAbs for 50 min. (see below), slides were treated sequentially with rabbit anti-mouse IgG, swine anti-rabbit IgG and rabbit anti-swine IgG, all biotin conjugated. Slides were then incubated with strepavidin-peroxidase and antibody localization performed using the diaminobenzidene reaction. Specimens obtained from Beth-Israel-Deaconess-Harvard Medical School were stained as previously described using an automated Ventana NexES instrument (Ventana Medical Systems, Tucson, AZ) (Magi-Galluzzi, C. et al., 1997). Antigen retrieval was done by microwave for 15 min. in EDTA, pH 8.05 at 750W. mAbs purified at a concentration of ~ 1ug/ul from SCID ascites were used at the following

concentrations: 1G8 = 1:20; 3E6 = 1:30; 2H9 = 1:50; 4A10 = 1:100; 3C5 = 1: 100. mAb 1G8 was produced in CellPharm System 100 and used at a concentration of 1:10. Positive controls included LAPC-9 and LNCaP-PSCA and negative controls were LNCaP and isotype-matched irrelevant antibody. Primary biopsy specimens were available for three patients with bone metastases. To approximate conditions of decalcification, slides from these specimens were treated for 20 min. in Decal-Stat (Lengers, NY) prior to staining with PSCA mAbs.

Monoclonal antibodies (mAbs) were raised against a PSCA-GST fusion protein lacking both the amino and carboxyl terminal signal sequences of PSCA. Positive fusions were selected by ELISA using the PSCA-GST fusion protein and GST alone. Out of 400 hybridomas screened, 28 recognized the PSCA-GST fusion but not GST alone. These fusions were screened secondarily by flow cytometry of nonpermeabilized 293T cells transfected with PSCA and mock transfected 293T cells. Secondary screening by FACS was done in order to select clones capable of recognizing PSCA on the cell surface, hypothesizing that these might later become useful for *in vivo* targeting applications. Seven positive fusions were identified in this manner (mAbs 2A2, 3G3, 4A10, 1G8, 3E6, 3C5 and 2H9), of which five (mAbs 4A10, 1G8, 3E6, 3C5 and 2H9) were subcloned and purified.

The mAbs were tested for their ability to immunoprecipitate PSCA and/or to recognize PSCA on immunoblots. All mAbs were able to immunoprecipitate PSCA from 293T-PSCA cells, as well as from LAPC-9 prostate cancer xenograft tumors that express high levels of endogenous PSCA (Figure 37). Likewise, all mAbs detected PSCA by immunoblotting, although mAbs 2H9 and 3E6 recognized only the ~ 12 kd deglycosylated form of PSCA (Figure 34).

The location on PSCA of the epitopes recognized by the five mAbs was determined by immunoblot analysis using three truncated PSCA-GST fusions proteins. mAbs 4A10, 2H9 and 3C5 recognize an epitope residing within the amino-terminal portion of PSCA (i.e., amino acids 21-50); mAb 1G8 recognizes an epitope within the middle region of

PSCA (i.e., amino acids 46-85); and mAb 3E6 reacts within the carboxyl-terminal portion of PSCA (amino acids 85-99) (Figure 15). All five mAbs are IgG as described in Figure 15. These results demonstrate that the five mAbs can detect PSCA in multiple assays and recognize at least three distinct epitopes on PSCA.

PSCA mAbs Stain the Cell Surface of Prostate Cancer Cells

The utility of mAbs for studying PSCA biology and for potential clinical applications such as *in vivo* targeting applications is dependent on their ability to recognize the antigen of interest on the plasma membrane (Liu, H. et al., 1997; McLaughlin, P. et al., 1998; Wu, Y. et al., 1995; Tokuda, Y. et al., 1996). In order to determine the ability of mAbs 2H9, 3E6, 1G8, 4A10 and C5 to recognize PSCA specifically on the cell surface of prostate cancer cells, LNCaP cells transfected with PSCA (LNCaP-PSCA) and LAPC-9 cells were examined by flow cytometry and indirect immunofluorescence. As with 293T-PSCA cells, all five mAbs were able to detect PSCA on the cell surface of nonpermeabilized LNCaP-PSCA and/or LAPC-9 cells by flow cytometry (Figure 33). Mock-transfected LNCaP and LNCaP transfected with a neomycin-alone containing vector (LNCaP-neo), neither of which expresses detectable PSCA mRNA, were both negative.

Immunofluorescent analysis was performed on both permeabilized and nonpermeabilized cells in order to ascertain whether PSCA protein localizes to the cell surface (Liu, H. et al., 1997). Nonpermeabilized LNCaP-PSCA showed clear cell surface reactivity with mAbs 1G8, 3E6, 4A10 and 3C5, but did not stain with mAb 2H9 (mAb 2H9 also did not detect PSCA on LNCaP-PSCA cells by FACS). LAPC-9 cells showed cell surface reactivity with all five mAbs (Figure 35). LNCaP-neo, as predicted, was negative both with and without permeabilization. Permeabilization of LNCaP-PSCA and LAPC-9 resulted in both membrane and cytoplasmic staining. All mAbs produced a punctate staining pattern on the cell surface, which was most pronounced with mAbs 3E6, 3C5 and 4A10 (Figure 35). This pattern may reflect aggregation or clustering of PSCA to

regions of the cell surface. These results demonstrate that all five mAbs react with PSCA on the cell surface of intact prostate cancer cells.

Immunohistochemical Staining of PSCA in Normal Prostate

PSCA mRNA localizes to a subset of basal cells in normal prostate, suggesting that PSCA may be a cell surface marker for prostate stem/progenitor cells (Reiter, R. et al., 1998). In order to test the possibility that PSCA protein may be a marker of basal cells, PSCA expression was examined immunohistochemically in paraffin-embedded sections of normal prostate. mAbs 1G8 and 2H9 stained the cytoplasm of both basal and secretory cells , while mAb 3E6 reacted predominantly with basal cells (Figure 38). Atrophic glands, which express basal cell cytokeratins, stained strongly with all three mAbs (Figure 38) (O'Malley, F. P. et al., 1990). mAbs 3C5 and 4A10 gave strong background staining and/or nonspecific nuclear staining in paraffin sections and were not used further. These results suggest that although PSCA mRNA is detected specifically in basal cells, PSCA protein can be detected in both epithelial cell layers (i.e. basal and secretory) of the prostate, although there are some differences in the staining patterns of individual antibodies.

Immunohistochemical Analysis of Normal Tissues

Our initial studies indicated that PSCA expression in men was largely prostate-specific, with low levels of detectable RNA in kidney and small intestine. PSCA mRNA was also detected in placenta. The prostate-specificity of PSCA protein expression was tested by immunohistochemical staining of 20 tissues using mAb 1G8 (see Table 1). Positive tissue staining with mAb 1G8 was confirmed with mAbs 2H9 and/or 3E6 in order to ensure reproducibility with mAbs directed against distinct epitopes. Staining was also performed and scored independently at two institutions in order to confirm the results. As predicted by the RNA analysis, placenta was positive with all mAbs tested, with cytoplasmic staining detected in the trophoblasts (Figure 39A). In kidney staining was detected in the

collecting ducts and distal convoluted tubules, but not in glomeruli (Figure 39A). Transitional epithelium of the bladder and ureter, which had not been examined previously at the mRNA level, was positive with all mAbs tested (Figure 39A). The only other tissue with significant immunoreactivity was colon, in which single cells deep within the crypts stained intensely positive (Figure 39A). Double staining with chromogranin indicated that these cells are of neuroendocrine origin.

In order to confirm that mAb reactivity in bladder represented PSCA, Northern blot analysis was performed on three normal bladder samples obtained at radical cystectomy and compared with PSCA expression in prostate, kidney and the LAPC-9 xenograft (Figure 39B). PSCA mRNA was detected in bladder at levels lower than those seen in prostate, confirming the immunohistochemical result. No signal was detected in the three kidney specimens, consistent with our previous observation that PSCA expression in kidney is significantly lower than prostate (Reiter, R. et al., 1998). LAPC-9, a prostate cancer xenograft established from a bone metastasis, expresses very high levels of PSCA mRNA compared with normal bladder and prostate (Whang, Y. E. et al., 1998). These results confirm that PSCA expression in men is largely prostate-predominant; however, there is also detectable PSCA protein expression in urothelium, renal collecting ducts and colonic neuroendocrine cells.

PSCA Protein is Expressed by a Majority of Localized Prostate Cancers

In our previous study, mRNA was expressed in ~ 80% of tumors and appeared to be expressed more highly in normal than malignant glands (Reiter, R. et al., 1998). In order to determine if PSCA protein can be detected in prostate cancers and if PSCA protein levels are increased in malignant compared with benign glands, paraffin-embedded pathological specimens of primary and metastatic prostate cancers were immunostained with mAb 1G8 (Figures 21 and 28). Isolated cases were also stained with mAbs 3E6 or 2H9 in order to confirm the specificity of the staining. Twelve of 15 primary cancers stained positive (Figure 21), including 2/2 cases containing foci of high grade prostatic intraepithelial neoplasia. Staining intensity varied, with 7 cases showing equivalent

staining in cancer and adjacent normal glands and 5 showing significantly stronger staining in cancer. In some cases there was strong expression in the malignant glands and undetectable staining in adjacent normal tissue (Figure 21; patient 1). Also, there were some cases in which staining was heterogeneous, with some malignant glands staining more strongly than others (Figure 21; patient 2). Overall, poorly differentiated tumors stained more strongly than well differentiated ones, suggesting that PSCA overexpression may correlate with increasing tumor grade (Figure 21; patient 3). These results demonstrate that PSCA protein is expressed in prostate cancer. Consistent with our previous mRNA in situ studies, PSCA appears to be overexpressed in a significant percentage of cancers, perhaps in concert with increasing tumor grade.

This study describes the first characterization of PSCA protein expression using five monoclonal antibodies directed against PSCA. Because these mAbs recognize epitopes on the exterior of the cell surface, they may have utility for prostate cancer diagnosis and therapy (Liu, H. et al., 1997). One possibility is that these mAbs could be used to locate sites of metastatic disease, similar to the Prostascint scan which uses an antibody directed against PSMA (Sodee, D. B. et al., 1996). Another possibility is that they may be used to target prostate cancer cells therapeutically, either alone or conjugated to a radioisotope or other toxin. Similar approaches are currently being evaluated using antibodies directed against extracellular epitopes on PSMA (Murphy, G. P. et al., 1998; Liu, H. et al., 1997; Liu, H. et al., 1998).

PSCA mAbs stain the cell surface in a punctate manner, suggesting that PSCA may be localized to specific regions of the cell surface. GPI-anchored proteins are known to cluster in detergent-insoluble glycolipid-enriched microdomains (DIGS) of the cell surface (Varma, R. and Mayor, S., 1998). These microdomains, which include caveolae and sphingolipid-cholesterol rafts, are believed to play critical roles in signal transduction and molecular transport (Anderson, R. Caveolae, 1993; Friedrichson, T. and Kurzchalia, T. V., 1998; Hoessli, D. C. and Robinson, P. J., 1998). Thy-1, a homologue of PSCA, has previously been shown to transmit signals to src kinases through interaction in lipid-microdomains (Thomas, P. M. and Samuelson, L. E., 1992; Stefanova, I. et al., 1991).

Preliminary subcellular fractionation experiments in our laboratory confirm the presence of PSCA in DIGS (Xavier, R. et al., 1998) .

GPI-anchored proteins have also been reported to localize to prostasomes, membrane-bound storage vesicles released by prostate epithelial cells (Ronquist, G. and Brody, I., 1985). CD59, a GPI-anchored inhibitor of complement-mediated cytolysis, is found in high concentrations in prostasomes of normal prostate epithelial cells and prostatic secretions (Rooney, I. et al., 1993). PSCA protein is detected in prostate secretory cells.

Contrary to our previous finding that PSCA mRNA localized exclusively to basal cells, the current results suggest that PSCA protein may be present in both basal and secretory cells. Similar differences between mRNA and protein localization in prostate have been described for PSMA and androgen receptor (Magi-Galuzzi, C. et al., 1997; Kawakami, M. and Nakayama, J., 1997). One possible explanation for the presence of PSCA protein in secretory cells is that PSCA mRNA is transcribed in basal progenitor cells but that PSCA protein expression persists as basal cells differentiate into secretory cells. Another possibility is that PSCA protein may be transferred from basal to secretory cells posttranslationally.

Differences in staining intensity of basal and secretory cells by mAbs 3E6, 1G8 and 2H9 may reflect the distinct epitopes recognized by the antibodies and/or differences in posttranslational modification of PSCA in basal and secretory cells. Supporting this possibility is the observation that the five mAbs do not react equally with PSCA in all assays or cell lines. mAb 2H9 recognizes PSCA on the cell surface of LAPC-9 but not LNCaP-PSCA, suggesting that the epitope recognized by this antibody may be altered or obscured in the latter cell type. We have also observed that mAb 3E6 does not stain cancers as strongly as mAbs 1G8 and 2H9 in some cases, suggesting that it may react with certain forms of PSCA preferentially.

Although largely prostate-specific in men, PSCA is also expressed at lower levels in urothelium, colonic neuroendocrine cells, and renal tubules and collecting ducts. The staining seen in renal tubules and collecting ducts is interesting in that these structures derive embryologically from the ureteric bud of the mesonephric duct, suggesting a possible reason for the staining patterns seen in kidney. The absence of detectable PSCA mRNA in kidney specimens may reflect either low levels of expression or the possibility that the samples were obtained primarily from the renal cortex, whereas the collecting ducts are located in the renal medulla.

The primary impetus for identifying prostate-specific cell surface genes is the desire to develop selective, nontoxic therapies. PSMA, another "prostate-restricted" protein, has also been shown to be expressed in duodenum, colonic neuroendocrine cells and proximal renal tubules (Silver, D. A. et al., 1997). Preliminary reports of PSMA vaccine therapy have not produced significant toxicity (Tjoa, B. A. et al., 1998).

Expression of PSCA in urothelium and kidney appears to be lower than in normal prostate and significantly less than that seen in many of the prostate cancers evaluated. Therapies directed against PSCA may therefore be relatively selective for cancer, much as Her-2/neu antibodies primarily target breast cancers that overexpress Her-2/neu (Disis, M. L. and Cheever, M. A., 1997).

Expression of PSCA in urothelium and kidney raises the possibility that it may be expressed in transitional and renal cell carcinomas. Two bladder cancers examined do express PSCA, one at levels similar to LAPC-9, suggesting that PSCA may be overexpressed in some cases of transitional cell carcinoma. A more complete survey of bladder cancer specimens will be required to test this possibility.

The data herein supports our earlier observation that PSCA is expressed in a majority of prostate cancers. Likewise, PSCA protein is overexpressed in some prostate tumors when compared to adjacent normal glands, supporting its use as a target for prostate cancer therapy. In contrast to the mRNA in situ studies, the current results suggest that PSCA

protein expression may correlate with cancer stage and/or grade. Similar differences between RNA and protein expression have been noted for thymosin Beta-15 (Bao, L., et al., 1996).

Table 1. PSCA expression in normal tissues.

Staining	Tissue
Positive	Prostate (epithelium)
	Bladder (transitional epithelium)
	Placenta (trophoblasts)
	Colon (neuroendocrine cells)
	Kidney (tubules and collecting duct)*
Negative	Kidney (glomeruli)
	Prostate (stroma)
	Bladder (smooth muscle)
	Testis
	Endometrium
	Small intestine
	Liver
	Pancreas
	Breast
	Gallbladder
	Skeletal muscle
	Brain
	Peripheral nerve
	Bone marrow
	Thymus
	Spleen
	Lung
	Bronchus
	Heart

* mAb 3E6 reacts with the distal convoluted tubule, while mAb 1G8 reacts with distal tubules and, in some cases, proximal tubules.
** subsequent experimental analysis also showed PSCA expression in normal stomach tissue.

Example 6:

PSCA expression in prostate cancer bone metastases

This experiment shows that PSCA expression is amplified in bone metastases of prostate cancer.

MATERIALS AND METHODS

Horse Serum (NHS) (GIBCO #26050-070) was diluted (1/20 dilution) in 1% Casein, PBST. The antibodies of the invention that recognize PSCA were diluted in 1/100 NHS, PBST.

The detection system included HRP-rabbit anti-mouse Ig (DAKO P260), HRP-swine anti-rabbit Ig (DAKO P217), HRP-rabbit anti-swine Ig (DAKO P164). Each were diluted 1/100 in 1/100 NHS, PBST.

3,3'-diaminobenzidine tetrahyrochloride (DAB) (Fluka) stock was made by dissolving 5 gm in 135 ml of 0.05 M Tris, pH 7.4. DAB was aliquoted into 1 ml/vial and frozen at – 20° C. A working solution of DAB was made by adding 1 ml of DAB to 40 ml of DAB buffer and 40 microliters of 50% H_2O_2.

DAB buffer was prepared by combining 1.36 gm Imidazole (Sigma #I-0125) with 100 ml D^2- H_2O, then adjusting the pH to 7.5 with 5 N HCl. After the pH adjustment 20 ml of 0.5 M Tris pH 7.4 and 80 ml of D^2- H_2O were added.

A section of a tissue/tumor known and previously demonstrated to be positive for the antibody was run with the patient slide. This slide served as a "positive control" for that antibody. A section of the patient's test specimen was incubated with a negative control antibody in place of the primary antibody. This slide served as a "negative control" for the test.

The staining procedure was as follows. Bone samples were applied to slides. The slides were then baked overnight at 60° C. Slides were deparaffinize in 4 changes of xylene for 5 minutes each and passed through a graded series of ethyl alcohol (100% x 4, 95% x 2) to tapwater then transferred to NBF, and fixed for 30 minutes. The fixed slides were placed in running tapwater for 15 minutes, transferred to 3% H_2O_2-MeOH, incubated for 10 minutes, and washed in running tapwater for 5 minutes, then rinse in deionized water.

Slides were then subjected to 0.01 M citrate buffer pH 6.0, heated at 45° C for 25 minutes, cooled for 15 min and then washed in PBS. The slides were then rinsed in PBS and placed onto programmed DAKO Autostainer, using the following four step program. The four step program is as follows. The slide is rinsed in PBS and blocked with 1/20 NHS in 1% Casein in PBST for 10 minutes. Primary antibody is then applied and incubated for 30 minutes followed by a buffer rinse. HRP-Rabbit anti-Mouse Ig is then applied and incubated for 15 minutes followed by another buffer rinse. HRP-Swine anti-Rabbit Ig is applied and incubated for 15 minutes followed by a buffer rinse. HRP-Rabbit anti-Swine Ig is applied and incubated for 15 minutes followed by a buffer rinse.

DAB is then applied to the slide and incubated for 5 minutes followed by a buffer rinse. A second DAB is applied and incubated for 5 minutes followed by a buffer rinse.

The slides are removed from the Autostainer and placed into slide holders, rinsed in tapwater and counter stained with Harris hematoxylin (15 seconds). The slide is then washed in tapwater, dipped in acid-alcohol, washed in tapwater, dipped in sodium bicarbonate solution, and washed in tapwater. The slides are then dehydrated in graded ethyl alcohols (95% x 2, 100% x 3) and Propar x 3 and coverslipped with Permount.

PSCA Protein is Expressed Strongly in Prostate Cancers Metastatic to Bone

Prostate cancer is unique among human tumors in its propensity to metastasize preferentially to bone and to induce osteoblastic responses. Nine sections of prostate cancer bone metastases were examined immunohistochemically (Figure 28). All reacted

intensely and uniformly with mAb 1G8 (and/or 3E6). In two instances, micrometastases not readily detectable on hematoxylin and eosin sections could be seen after staining with mAb 1G8 (Figure 28; patient 5). Overall, staining in bone metastases was stronger and more uniform than in the primary tumors. In three cases, biopsy specimens from the primary tumors were available for comparison. All were weakly positive for PSCA when compared with the matched bone metastasis, suggesting that PSCA expression was increased in bone. In one biopsy specimen, weak staining was present in only a small focus of malignant glands, while the remaining tumor was negative (Figures 21 and 28; Patient 4). In two cases, the biopsy specimens were obtained 10 and 15 years prior to the bone metastasis, indicative of a long latency period between the development of the primary and metastatic lesions. To rule out the possibility that the strong staining in bone was caused by the decalcification process used to prepare bone sections, the three primary biopsy specimens were also treated with decalcification buffer. Although this treatment increased background staining, it did not alter epithelial reactivity significantly, indicating that the strong signal in bone was unlikely to be caused by the decalcification process. These results suggest that PSCA may be selected for or upregulated in prostate cancer metastases to bone.

FIGS. 21-23 show the bone samples of bone metastases of prostate cancer were positive for PSCA. Nine sections of prostate cancer bone metastases were examined. Consistent, intense staining was seen in nine prostate cancer bone metastases and all reacted intensely and uniformly with mAb 1G8 (and/or 3E6). In two instances, the pathologist could not readily identify the metastasis until staining with 1G8 highlighted the lesion. Overall, staining in bone metastases appeared stronger and more uniform than in the primary tumors.

These results suggest that PSCA may be greatly overexpressed in prostate cancer metastases to bone. This is particularly interesting since Sca-2, a close homologue of PSCA, was recently reported to suppress osteoclast activity in bone marrow. If PSCA had similar activity, it might provide one explanation for the tendency of prostate cancer metastases to produce an osteoblastic response, since inhibition of osteoclast activity would tilt the balance of activity in bone to bone formation. Another possibility is that PSCA might be involved in adhesion to

bone, since other Ly-6/Thy-1 family members are involved in similar processes. There was heterogeneous expression of PSCA in a number of primary prostate cancers. These results further support the use of PSCA as a novel target for advanced disease.

One of the most intriguing results of the present study was the consistent, intense staining seen in the nine prostate cancer bone metastases. LAPC-9, a xenograft established from a bony metastasis, also stained intensely for PSCA. In three patients, matched primary biopsy specimens showed low levels of PSCA expression compared to the bony metastases. Areas of strong PSCA expression in the primary tumors of the three patients examined may have been missed since only biopsies were available for analysis. Heterogeneous expression of PSCA was detected in at least one matched primary tumor, as well as in number of primary tumors for whom matched metastatic lesions were not available. Also, in two cases the primary tumor was sampled at least a decade prior to the bone metastasis, raising the possibility that clones expressing high levels of PSCA within the primary could have developed subsequent to the initial biopsy. These results clearly demonstrate PSCA expression in bone metastases, further supporting it as a novel target for advanced disease.

Example 7:

PSCA overexpression in bladder and pancreatic carcinomas

This experiment shows that PSCA expression is higher in bladder carcinomas than normal bladder.

Tissues from prostate, bladder, kidney, testes, and small intestine (including prostate cancer and bladder and kidney carcinomas) were obtained from patients. These tissues were then examined for binding to PSCA using northern and western blot analyses as follows.

For northern blot analyses, tissue samples were excised and a less than 0.5 x 0.5 cm tissue sample was quick frozen in liquid nitrogen. The samples were homogenized in 7

mls of Ultraspec (Biotecx, Houston, Texas), using a polytron homogenizer using the protocol provided by Biotecx (Ultraspec™ RNA Isolation System, Biotecx Bulletin No:27, 1992).

After quantification, 20 μg of purified RNA from each sample were loaded onto a 1% agarose formaldehyde gel. Running and blotting conditions were the same as was used in Example 1. The filters were separately probed with labeled PSCA and an internal control, actin. Filters were washed and exposed for several hours-overnight.

For western blot analyses, tissue samples were excised and a less than 0.5 x 0.5 cm tissue sample was taken and quickly minced and vortexed in equal volume of hot 2X Sample Buffer (5%SDS, 20% glycerol). Samples were incubated at 100° for 5 mins, vortexed and clarified for 30 min. Protein concentrations were determined by Biorad's DC Protein Assay kit (Richmond, CA). 40μg/sample was loaded on a 12% polyacrylamide protein gel. Transfer to a nitrocellulose filter was done by standard methods (Towbin et al. PNAS 76:4350 (1979). A western blot was performed by incubating the filter with IG8 primary antibody followed by a secondary antibody, i.e., a goat αmouse IgG HRP. Detection was by Amersham ECL Detection kit (Arlington Heights, IL).

1G8 recognized and bound the PSCA on the cells surface of LAPC9 and a bladder carcinoma (designated bladder (Rob)) in a western blot analysis (FIG. 6). In FIG. 6, all tissues except LAPC9 were normal. A northern blot analysis confirmed elevated PSCA in the bladder carcinoma tissue (designated bladder (Rob) (also referred to as Rob's Kid CA) and LAPC9) (FIG. 25).

A Northern blot analysis was performed, testing transcripts isolated from pancreatic cancer cell lines: PANC-1 (epithelioid, ATCC No. CRL-1469), BxPC-3 (adenocarcinoma, ATCC No. CRL-1687), HPAC (epithelial adenocarcinoma, ATCC No. CRL-2119), and Capan-1 (adenocarcinoma, liver metastasis, ATCC No. HTB-79). The Northern blot was probed with a full length cDNA clone of PSCA which detected PSCA transcripts in two pancreatic cancer cell lines, HPAC and Capan-1 (FIG. 63).

A Western blot analysis using the PSCA mAb 1G8 detected high levels of PSCA protein in the HPAC cell line and lower levels in Capan-1 and ASPC-1 (adenocarcinoma, ascites, ATCC No. CRL-1682) (FIG. 64).

Example 8:

PSCA gene amplification in prostate cancer

This experiment shows that PSCA gene copy number is increased similar to an increase in copy number of c-myc (Figure 17). This is important because c-myc amplification correlates with poor outcome. Thus, the data suggests that PSCA amplification may also be a predictor for poor outcome.

FISH with Chromosome Enumeration Probes and a Probe for c-Myc.

The method of FISH is well known (Qian, J. et al., "Chromosomal Anomalies in Prostatic Intraepithelial Neoplasia and Carcinoma Detected by Fluorescence *in vivo* Hybridization," *Cancer Research,* 1995, 55:5408-5414.) Briefly, tissue sections (samples 34 and 75 were from two patients) were deparaffinized, dehydrated, incubated in 2X SSC at 75°C for 15 min, digested in pepsin solution [4mg/ml in 0.9% NaCl (pH 1.5)] for 15 min at 37°C, rinsed in 2X SSC at room temperature for 5 min, and air-dried.

Directly labeled fluorescent DNA probes for PSCA and for the 8q24 (c-myc) region were chosen. The PSCA cDNA (SEQ ID NO:1) (Fig. 1) was used to identify a 130 kb bacterial artificial chromosome (bac) clone (PSCA probe) that in turn was used in the FISH analysis in accordance with the manufacturer's protocol (Genome Systems Inc.) The bac clone so identified and used in the FISH analysis was BACH-265B12 (Genome Systems, Inc. control number 17424).

Dual-probe hybridization was performed on the serial 5-μm sections using a SG-labeled PSCA probe together with a SO-labeled probe for 8q24 (c-myc). Probes and target DNA

were denatured simultaneously in an 80°C oven for 5 min. and each slide was incubated at 37°C overnight.

Posthybridization washes were performed in 1.5 M urea/0.1X SSC at 45°C for 30 min and in 2X SSC at room temperature for 2 min. Nuclei were counter-stained with 4.6-diamidino-2-phenylindole and anilfade compound *p*-phenylenediamine.

The number of FISH signals was counted with a Zeiss Axioplan microscope equipped with a triple-pass filter (I02-104-1010; VYSIS). The number of c-myc signals and PSCA signals were counted for each nucleus, and an overall mean c-myc:PSCA ratio was calculated. Results are shown in Figure 17.

The results show that PSCA gene copy number increased in prostate cancer samples (Figure 17). The PSCA gene is located at 8q24.2. The increase in gene copy number is due to both a gain in chromosome 8, and amplification of the PSCA gene (Figure 17). Interestingly, the increase in PSCA gene copy number is similar to an increase in gene copy number of c-myc (Figure 17) which is also located at 8q24. A previous study has demonstrated that a gain of chromosome 8 and amplification of c-myc are potential markers of prostate carcinoma progression (R B Jenkins *et al* 1997 *Cancer Research* 57: 524-531).

Example 9:

Reporter gene construct using the hPSCA 9 kb upstream region to drive luciferase expression

The 14 kb Not I genomic fragment encoding the human PSCA gene was isolated from λFIXII library encoding human genomic DNA (Stratagene), by screening the library with a full length human PSCA cDNA probe, as described in example 4 (Sambrook et al., 1989, Molecular Cloning (Cold Spring Harbor). The 14 kb human PSCA genomic fragment includes 9 kb of PSCA upstream sequences that was used to drive expression of a reporter gene.

The reporter gene vectors are depicted in Figure 42 and were constructed as follows. The 14 kb Not I fragment was sub-cloned from the λ vector into a Bluescript KS vector (Stratagene), resulting in the pBSKS-PSCA (14kb) construct. The PSCA upstream sequence was subcloned from pBSKS-PSCA (14 kb) by PCR amplification using a primer corresponding to the T7 sequence contained within the Bluescript vector, and a primer corresponding to a sequence contained within PSCA exon 1 (primer H3hPSCA3'-5, the sequence of this primer is as follows: The sequence of H3hPSCA3'-5 is 5'-gggaagcttgcacagccttcagggtc-3' (SEQ ID NO. 24). The primer corresponding to PSCA exon 1 contained an introduced HindIII sequence to allow further subcloning following PCR amplification. The resulting amplified fragment was digested with HindIII and was subcloned into the pGL3-basic vector (Promega) to generate pGL3-PSCA (7 kb) which was used to generate a series of deletion reporter gene constructs containing varying lengths of PSCA upstream sequences operatively linked to the luciferase gene (Figure 42). The deleted portions of the PSCA upstream regions were obtained by subcloning restriction fragments from pGL3-PSCA (7 kb). The PSCA upstream region between –9 kb and –7 kb was subcloned from the pBSKS-PSCA (14 kb) construct, the Not I site was converted into a blunt end by Klenow and the fragment was cloned into the SacI/HindIII sites of pGL-PSCA (7 kb) in order to obtain the pGL3-PSCA (9 kb) construct. The reference to the sequences upstream of the PSCA coding region, such as –9 kb and –6 kb (etc.), are relative to the ATG start translation codon. The reporter gene constructs pGL3-PSCA (9 kb), pGL3-PSCA (6 kb), pGL3-PSCA (3 kb), and pGL3-PSCA (1 kb) were operatively linked to the luciferase gene (Figure 42). Plasmid, pGL3-CMV contains the cytomegalovirus promoter (Boshart, M. et al., 1985 *Cell* 41:521-530) linked to the luciferase gene and was used as a positive control. Also, plasmid pGL3 contains no promoter sequence and was used as a negative control plasmid.

Example 10:

Transfection assay using a reporter gene construct containing the hPSCA upstream region.

Triplicate dishes of prostate and non-prostate cell lines were transfected by Tfx50 (Boeringer Manheim) with the PSCA construct pGL3-PSCA (9 kb), or the positive control construct, pGL3-CMV both described in Example 9 above, and assayed for luciferase activity (Figure 43). The cells and cell lines transfected include PrEC (androgen-independent prostate basal cell), LNCaP (androgen-dependent prostate secretory cell line), LAPC4 (androgen-dependent prostate cell line), HT1376 (bladder cell line), and 293T (kidney cell line). Expression activities of the constructs are expressed as a percentage of the activity of the CMV promoter. Standard errors are indicated above the bars.

The results show that 9 kb of human PSCA upstream sequences drives expression of the luciferase gene in a tissue-specific manner similar to the mRNA expression patterns seen for native hPSCA shown in Figure 10 (Example 1). Luciferase was readily detectable in both androgen-dependent and androgen-independent prostate cell lines and bladder. Luciferase was also detectable, although at a lower level, in kidney cells.

Example 11:

Identification of regulatory elements within the PSCA upstream region

Triplicate dishes of PrEC (Clonetech) or LNCaP cells were transfected with the reporter gene constructs or the positive control construct described in Example 9 above, and assayed for luciferase activity. The reporter gene constructs comprise various lengths of the hPSCA upstream region operatively linked to the luciferase gene. The positive control construct, pGL3-CMV, comprises the CMV promoter operatively linked to the luciferase. The cells were transfected using a Tfx50 transfection system (Promega). Expression of luciferase in the transfected cells were assayed using a Dual Luciferase Reporter Assay System (Promega), and the level of luciferase expression was measure a relative luciferase unit (RLU).

The ability of the various lengths of the hPSCA upstream region to drive luciferase expression are expressed as a percentage of the activity of the positive control construct containing the CMV promoter. Standard errors are indicated.

The results shown in Figure 44 demonstrate that 3 kb of hPSCA upstream sequences drives expression of luciferase in both PrEc and LNCaP cells, but the level of detectable luciferase is 6 times higher in the LNCaP cells compared to the PrEC cells. This comparison was based on the level of detectable luciferase. In contrast, 1 kb of hPCSA upstream sequences did not drive expression of luciferase in either cell line.

Example 12:

A Targeting vector

A targeting vector was designed to delete the endogenous PSCA coding region, by homologous recombination. Figure 40 depicts a targeting vector for the mouse PSCA gene, and the strategy for using the targeting vector to delete the endogenous PSCA gene contained in a mouse cell. A targeting vector comprising a 12 kb SpeI fragment containing mouse PSCA upstream sequences, a NotI/EcoRI fragment containing the PGK promoter operatively linked to a neo^r gene from the pGT-N29 vector (New England BioLabs), and a 3.5 kb BstXI/XhoI fragment containing mouse PSCA downstream sequences. Constitutive expression of the neomycin resistance gene is controlled by the PGK promoter, and allows antibiotic selection of the targeted cells that contain the targeting vector.

As understood by one skilled in the art, the targeting vector described here includes but is not limited to the neo^r gene for selection of the cells that contain the targeting vector or can contain no selectable reporter gene. The targeting vector can also be used to generate transgenic mice, known in the art as knock-in or knock-out mice, depending on whether the targeting vector contains a reporter gene or not, respectively. The transgenic mice

can be used as an animal model to study the function of the PSCA gene in prostate development of mice.

As an example that is not intended to be limiting, the targeting vector was used to delete the wild type endogenous genomic mouse PSCA coding sequences in embryonic stem cells (ES) cells to generate cells that are heterozygous, containing a deleted PSCA gene. For example, the heterozygous cells generated using the targeting vector are PSCA+/neo^r as shown by the results in Figure 40. The phenotype of the heterozygous cells or transgenic mice can be compared with that of wild type PSCA cells or animals.

The targeting vector was constructed as follows. The ends of the 12 kb SpeI fragment containing the PSCA upstream and part of exon 1 sequences was blunt-ended and linked to the blunt-ended NotI/EcoRI fragment from pGT-N29 (BioLabs) containing the neomycin-resistance gene. The 3' end of the neomycin-resistance gene was linked to a blunt-ended 3.5 kb BstXI/XhoI fragment containing part of PSCA exon 3 and the downstream sequences. The resulting fragment was cloned into pGT-N29 to generate the targeting vector pGT-N29-mPSCA5'/3'.

The targeting vector was transfected into ES cells by electroporation using the method described in the following: Teratocarcinomas and Embryonic Stem Cells; A Practical Approach. IRL Press, Oxford (1987). Neomycin-resistant cells were selected and genomic DNA was isolated from the selected cells. A genomic Southern analysis was performed to determine the outcome of the homologous recombination reaction. 10 μg of DNA from the homologous recombination reaction and non-targeted ES cells were digested with EcoRI and analyzed by the Southern blot method (Southern, EM 1975 J. Molec. Biol. 98:503). The blot was probed with a XhoI/EcoRI fragment that contains sequences 3' to the PSCA coding region. The results show that the probe detects a 10 kb fragment that corresponds to the control non-targeted cells that are PSCA+/PSCA+, and a 4 kb fragment that corresponds to the targeted cells that are heterozygous and contain PSCA+/neo^r.

Example 13:

Transgenic mouse models for prostate cancer

The present invention contemplates a strategy to generate transgenic mouse models for prostate cancer, using the upstream regions of the PSCA gene to drive expression of an oncogene, to induce tumor formation in prostate basal cells. As shown in Figure 41, the strategy involves administration, e.g., microinjection, of a chimeric oncogene vector, comprising the upstream region of the PSCA gene operatively linked to a transgene that encodes a gene product that induces formation of a tumor. Other researchers have used this technique, using different prostate and non-prostate regulatory sequences operatively linked to an oncogene. For example, C3(1) is a prostate-predominant regulatory sequence (Moroulakou *et al* 1994 Proc. Nat. Acad. Sci. 91: 11236-11240) and probasin is a prostate-specific regulatory sequence (Greenberg *et al* 1995 Proc. Nat. Acad. Sci. 92: 3439-3443), and both of these regulatory sequences drive expression of a transgene in prostate secretory cells. Cryptdin2 is a small-intestine predominant regulatory sequence (Garagenian *et al* Proc. Nat. Acad. Sci. 95: 15382-15387) that caused expression of an oncogene in prostate endocrine cells. In contrast, the present invention contemplates using the PSCA upstream region to drive expression of an oncogene in prostate basal cells, in order to generate a transgenic mouse model for prostate cancer.

The clinical characteristics of the induced prostate tumor can be analyzed and compared with known characteristics of tumors caused by the particular oncogene used in constructing the chimeric oncogene vector. In addition, various tissues and organs of the transgenic mouse can be analyzed by DNA, RNA and proteins analyses to ascertain the presence and expression patterns of the chimeric oncogene vector.

Example 14:

Transgenic mice carrying chimeric vectors comprising hPSCA upstream sequences and a transgene

The expression patterns of transgenes under the control of hPSCA upstream regions will be tested. Toward this end, chimeric mice carrying chimeric vectors comprising hPSCA upstream sequences and a transgene have been generated. Chimeric vectors comprising 9 kb or 6 kb of hPSCA upstream sequences operatively linked to a transgene were constructed, and are schematically represented in Figure 45. The transgenes included green fluorescent protein cDNA (GFP, Clontech) linked to the SV40 polyadenylation sequence (PSCA (9 kb)-GFP and PSCA (6 kb)-GFP), green fluorescent protein cDNA linked to a 3' region of the human growth hormone that contains an intron cassette that confers stability to mRNA (PSCA (9 kb)-GFP-3'hGH and PSCA (6 kb)-GFP-3'hGH) (Brinster *et al* 1988 PNAS 85: 836-840), and the genomic fragment encoding SV40 small and large T antigen including an intron (PSCA (9 kb)-SV40TAG and PSCA (6 kb)-SV40TAG) (Brinster *et al* 1984 Cell 37:367-379).

The chimeric vectors were used to generate a line of founder transgenic mice. Linearized chimeric vectors were microinjected into fertilized mouse eggs derived from intercrosses of C57BL/6X C3H hybrid mice. Founder mice that carried the chimeric vector were identified by Southern analysis of tail DNA, using GFP cDNA or SV40 genomic DNA as a probe. The number of founders of each transgenic mouse line is indicated on the right panel of Figure 45.

Example 15:

hPSCA upstream sequences drives expression of transgene in transgenic mice

Two independent founder mice carrying PSCA (9 kb)-GFP transgene were bred to Balb/c mice to obtain their offspring. At age of 8 weeks and 12 weeks, male and female transgenic or non-transgenic littermates were sacrificed. After sacrifice, all urogenital and other tissues were tested for GFP expression by observing the fixed tissues under fluorescent illumination. The results shown in Figure 46 show green fluorescent images of prostate, bladder and skin tissues from a non transgenic and a transgenic mouse. One

out of two founder lines expressed GFP protein in prostate, bladder and skin (Figure 46). Tissues that did not express GFP include: seminal vesicle, liver, stomach, kidney, lung, brain, testis, pancreas, heart, skeletal muscle, small intestine, colon, placenta.

Example 16:

Transcript expression pattern of PSCA in human and mouse tissue

The upper panel of Figure 47 shows a human multiple tissue Northern blot (obtained from Clonetech), probed with a full length human PSCA cDNA probe. The results demonstrate that human PSCA transcripts are abundant in prostate, and less abundant but readily detectable in placenta, but not detectable in spleen, thymus, testis ovary, small intestine, colon, peripheral blood leukocytes (PBL), heart, brain, lung, liver, muscle, kidney and pancreas.

The lower panel of Figure 47 shows an ethidium bromide-stained agarose gel of RT-PCR analysis of murine PSCA transcript expression patterns in various mouse tissues. The RT-PCR was prepared using Ultraspec.RNA (Biotex), and cDNA cycle kit (Invitrogen). Primers corresponding to a region within exon 1 and exon 3 of PSCA were used to amplify a 320 bp fragment. The exon 1 primer sequence is as follows: 5' primer: 5'-TTCTCCTGCTGGCCACCTAC-3' (SEQ ID NO:8). The exon 3 primer sequence is as follows: 3' primer: 5'-GCAGCTCATCCCTTCACAAT-3' (SEQ ID NO:9). As a control, to demonstrate the integrity of the RNA samples isolated from the various mouse tissues, a 300 bp G3PD fragment was amplified.

The results shown in the lower panel of Figure 47 demonstrate that murine PSCA transcripts are detectable in dorsal/lateral prostate, ventral prostate, bladder, stomach (cardiac, body and pyloric), and skin. In contrast, murine PSCA transcripts are not detectable in anterior prostate, ventral prostate, seminal vesicle, urethra, testis, kidney, duodenum, small intestine, colon, salivary gland, spleen, thymus, bone marrow, skeletal

muscle, heart, brain, eye, lung and liver. The G3PDH results demonstrate that the transcripts isolated from various mouse tissue were intact.

Example 17:

Immunohistochemical evidence of high level overexpression of PSCA in bladder cancer

The following example demonstrates that PSCA protein is highly overexpressed in various grades of bladder carcinoma as determined by immunohistochemical staining of paraffin-embedded bladder and bladder carcinoma tissue sections using PSCA mAb 1G8. Specifically, the following four tissues were examined: (A) normal bladder, (B) non-invasive superficial papillar, (C) carcinoma in situ (a high grade pre-cancerous lesion, (D) invasive bladder cancer.

The results are shown in FIG. 62. PSCA is expressed at low levels in the transitional epithelium of normal bladder tissue. Very high level expression was detected in the carcinoma in situ sample, in all cell layers. In the invasive bladder carcinoma sample, very strong staining was seen, again in all cells. Lower level staining was observed in the superficial papillar sample. These results suggest that PSCA expression levels may correlate with increasing grade.

In addition to the above study, preliminary results from an immunohistochemical analysis of PSCA expression in a large number of bladder and bladder carcinoma tissue specimens indicates the following (1) normal bladder expresses low levels of PSCA in the transitional eptihelium; similar levels of expression are seen in low grade, papillary, noninvasive lesions; (2) carcinoma in situ, a high grade, often quite aggressive precancerous lesion, is almost always (90%) intensely positive for PSCA in all cells; (3) PSCA is expressed intensely by ~30% of invasive cancers, i.e. overexpressed when compared to normal bladder; and (4) metastases are intensely positive for PSCA.

Example 18:

PSCA monoclonal antibody mediated inhibition of prostate tumors in vivo

The following examples demonstrate that unconjugated PSCA monoclonal antibodies inhibit the growth of human prostate tumor xenografts grown in SCID mice, both when administered alone or in combination.

A. Tumor inhibition using multiple unconjugated PSCA mAbs – Study 1

MATERIALS AND METHODS

Anti-PSCA Monoclonal Antibodies:

Murine monoclonal antibodies were raised against a GST-PSCA fusion protein comprising PSCA amino acid residues 18-98 of the PSCA amino acid sequence (SEQ ID NO:2) (FIG. 1B) and expressed in E. coli, utilizing standard monoclonal antibody production methods. The following seven anti-PSCA monoclonal antibodies, produced by the corresponding hybridoma cell lines deposited with the American Type Culture Collection on December 11, 1998, were utilized in this study:

Antibody	Isotype	ATCC No.
1G8	IgG1	HB-12612
2H9	IgG1	HB-12614
2A2	IgG2a	HB-12613
3C5	IgG2a	HB-12616
3G3	IgG2a	HB-12615
4A10	IgG2a	HB-12617
3E6	IgG3	HB-12618

Antibodies were characterized by ELISA, Western blot, FACS and immunoprecipitation for their capacity to bind PSCA. FIG. 49 shows epitope mapping data for the above seven anti-PSCA mAbs as determined by ELISA and Western analysis, as described in the accompanying figure legend, demonstrating that the seven antibodies recognize different epitopes on the PSCA protein. Immunohistochemical analysis of prostate cancer tissues and cells with these antibodies is described in Examples 5 and 6 infra.

Antibody Formulation:

The monoclonal antibodies described above were purified from hybridoma tissue culture supernatants by Protein-G Sepharose chromatography, dialyzed against PBS, and stored at -20°C. Protein determinations were performed by a Bradford assay (Bio-Rad, Hercules, CA).

A therapeutic antibody cocktail comprising a mixture of the seven individual monoclonal antibodies, as indicated in Table 2, below, was prepared and used for the treatment of SCID mice receiving subcutaneous injections of LAPC-9 prostate tumor xenografts. Mouse IgG, purchased from ICN (Costa Mesa, CA) was used as non-specific control antibody. Prior to injection into mice, all antibodies were sterilized using a 0.22-micron filter.

TABLE 2: Anti-PSCA Antibody Cocktail

Monoclonal Antibody	Isotype	Amount (% of total)
1G8	IgG1	2.0 mg (16.7%)
2H9	IgG1	1.0 mg (8.3%)
2A2	IgG2a	2.5 mg (20.8%)
3C5	IgG2a	2.0 mg (16.7%)
3G3	IgG2a	2.5 mg (20.8%)
4A10	IgG2a	1.5 mg (12.5%)
3E6	IgG3	0.5 mg (4.2%)

Introduction of Prostate Cancer Xenografts into SCID Mice:

The human prostate cancer xenograft line LAPC-9, which expresses very high levels of PSCA, was used to produce tumors in SCID mice (PCT Application No. WO98/16628, supra; Klein et al., 1987, supra).

For injection into IcR-SCID mice (Taconic Farms, Germantown, NY), a single-cell suspension of LAPC-9 was prepared as follows. An LAPC-9 xenograft tumor of approximately 2.0 g in size was harvested from a SCID mouse, minced into very small pieces using scissors and forceps, washed once in RPMI, and digested in a 1% solution of pronase for 20 minutes. After digestion, the cell suspension was washed twice in RPMI, and resuspended in 10 ml of PrEGM medium (Clonetics, Walkersville, MD). After overnight incubation, the cells were harvested and washed once in PrEGM, then passed through a 200-micron nylon filter to remove large clumps and debris. Cells passing through the filter were collected, centrifuged, and resuspended in PrEGM medium. Cells were then counted, and the appropriate number of cells was transferred to a new tube, centrifuged, and resuspended at 2X concentration in RPMI. An equal volume of ice cold Matrigel was then added to the cell suspension, and the suspension was kept on ice prior to injection. For injection, male IcR-SCID mice were shaved on their flanks, and each mouse received a single subcutaneous (s.c.) injection of 1×10^6 cells in a volume of 100 µl on the right flank. Mice injected with tumor cells were treated with either control antibodies or the anti-PSCA monoclonal antibody preparation as described below.

Treatment Protocol:

Twenty SCID mice injected with tumor cells were treated with either control antibodies (mouse IgG) or the anti-PSCA monoclonal antibody cocktail (above) as follows. Ten mice were treated with mouse IgG control antibody and ten mice were treated with the anti-PSCA monoclonal antibody preparation. Injections of 200 µg of the mouse IgG control antibody or the anti-PSCA monoclonal antibody cocktail were administered intraperitoneally on days -1, +3, +7, +11, +14, and +21 relative to the injection of the

tumor cells. Growth of LAPC-9 tumors was followed by caliper measurements to determine tumor volumes on days +32, +35, +39, +42, +47, +54 and +61 relative to injection of tumor cells. In addition, mice were periodically bled for assaying circulating PSA levels using a commercially available PSA test (American Qualex, San Clemente, CA).

One of the mice in the control group (mouse #2) expired during the course of the study and had no detectable tumor at the time.

RESULTS

SCID mice receiving a subcutaneous injection of the LAPC-9 prostate cancer xenograft were treated with either the anti-PSCA mAb preparation or mouse IgG control antibody, as described above. Palpable tumors first appeared in the mouse IgG control group at 4 weeks after tumor cell injection. Tumor volume measurements were initiated on day +32.

The results, which are tabulated in Table 3, below, as well as presented graphically in FIG. 48, show that all of the control mAb-treated mice developed tumors (9 out of 9 surviving, mouse #1, #3-10), but that none of the anti-PSCA mAb treated mice developed any detectable tumor growth (0 out of 10, mouse #11-20). The control-treated animals developed significant tumors rapidly in most instances, and these mice experienced constant tumor growth leading to progressively larger tumor sizes with time. By day 54, all control-treated mice had developed detectable tumors. In sharp contrast to the control-treated group, none of the ten mice treated with the anti-PSCA mAb preparation developed detectable tumors, even after 61 days post xenograft injection.

TABLE 3: Recorded tumor volume (mm^3) measurements

	DAYS						
MOUSE #*	32	35	39	42	47	54	61
1	416°	576	578	720	810	1045	1080
2	0	0	0	0			
3	100	269.5	450	476	544	648	810
4	0	0	0	0	0	87.5	151.3
5	338	420	800	900	1087	1265	2002
6	216	250.3	504	476	612	850.5	1050
7	252	472.5	637.5	720	720	720	1306
8	336	532	560	693	1080	1365	1617
9	0	160.9	225	294	478	640	900
10	0	0	195	294	341	504	769.5
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0

* Mice # 1-10 represent the group treated with the mouse IgG control antibody. Mice # 11-20 represents the group treated with the anti-PSCA mAb cocktail.

* Tumor volume corresponds to length (L) x width (W) x height (H) measurements in mm. To determine the ellipsoid volume represented in Figure 1, which accurately represents tumor mass (Tomayko and Reynolds, 1989), we used the formula L x W x H x 1/2.

Clinically, the control treated mice all displayed visual symptoms of progressively poor health as tumors developed and expanded. In contrast, the mice in the anti-PSCA mAb treatment group remained active, vigorous, and generally healthy in appearance throughout the treatment period, suggesting no apparent toxicity or obvious side-effects were associated with the treatment.

In addition to tumor volume, mice were bled for determination of circulating PSA. Circulating PSA levels correlated with increasing tumor volumes in the control group, whereas no detectable PSA was observed in the anti-PSCA mAb treated group throughout the experiment.

B. Tumor inhibition using multiple unconjugated PSCA mAbs – Study 2

To verify the results described in Example 18, supra, a newly prepared anti-PSCA mAb cocktail was evaluated for growth inhibition of LAPC-9 tumor xenografts in vivo, essentially as described above. Briefly, a new batch of each mAb was prepared and mixed together according to the proportions presented in Table 4 . All antibodies were tested for PSCA reactivity. SCID mice received a subcutaneous injection of LAPC-9 xenograft cells as described above. The mice were treated with either a cocktail of anti-PSCA mAb, or control preparations of mouse IgG or purified bovine IgG. A bovine IgG control group was included in this study in order to study the effect of bovine IgG co-purified with the anti-PSCA antibodies on protein G-sepharose. Two hundred micrograms of antibody was administered to each mouse by intraperitoneal injection on days -1, +3, +7, +11, +14, and +21 relative to the injection of the tumor cells. Tumor volume corresponding to length (L) x (W) x (H) in mm was monitored by caliper measurements, and serum was collected at weekly intervals. To determine the ellipsoid volume of the tumors, which accurately represents tumor mass, we used the formula L x W x H x 1/2 (Tomayko and Reynolds, 1989).

TABLE 4: Anti-PSCA antibody cocktail 2

Monoclonal Antibody	Isotype	Amount (% of total)
1G8	IgG1	8.0 mg (16.7%)
2H9	IgG1	4.0 mg (8.3%)
2A2	IgG2a	10.0 mg (20.8%)
3C5	IgG2a	8.0 mg (16.7%)
3G3*	IgG2a	10.0 mg (20.8%)
4A10	IgG2a	6.0 mg (12.5%)
3E6	IgG3	2.0 mg (4.2%)

* One of the monoclonal antibody preparations used to formulate this cocktail, 3G3, demonstrated weak reactivity.

The results of this study are presented in FIG. 53 and confirm the results generated from the study described in Example 18-A, supra. Animals in the anti-PSCA treated group experienced significant inhibition of tumor cell growth compared with both of the control groups. No detectable difference in tumor growth was observed in mice that received either bovine IgG or murine IgG. The tumors in the control groups grew at equal rates and with similar latency. In contrast, LAPC-9 tumors in mice receiving the anti-PSCA antibody cocktail exhibited a longer latency, a significantly slower rate of growth and smaller sizes at the end of the experiment. The average tumor volume at the final time point was 1,139 mm^3 for mice treated with murine IgG (day 46), 1091 mm^3 for mice treated with bovine IgG (day 42) and 391 mm^3 for anti-PSCA treated mice (day 46). Due to the large tumor sizes in the bovine IgG treated group, these mice were sacrificed earlier than mice in the other groups. In addition, tumor volume correlated with PSA levels in the serum of the treated mice. Some mice receiving anti-PSCA antibodies showed very small tumors or no tumor growth at all, as was previously observed in the study described in Example 1, supra. No apparent toxicity was associated with administration of any of this antibody cocktail preparation, consistent with the study described in Example 18-A.

C. Tumor inhibition in vivo using single unconjugated PSCA mAbs

Materials and Methods:

Several of the monoclonal antibodies described herein were studied for their ability to inhibit the growth of prostate tumor xenografts in their unconjugated (or, "naked") form using the previously described tumor challenge assay (see Examples 18-A and 18-B, above). Generally, the studies were conducted as described above, with slight modifications as described in the results sections presented below for each of the antibodies assayed.

C1: PSCA mAb 1G8

Anti-PSCA monoclonal antibody 1G8 is an IgG1 isotype antibody. The antitumor effect of 1G8 was evaluated using the LAPC-9 xenograft and mouse IgG as a control. The results presented in FIG. 54 demonstrate that treatment of mice with the 1G8 antibody inhibited tumor growth. Specifically, the average tumor volume at the final time point for the control group was 854 mm^3 versus an average tumor volume of 335 mm^3 for the 1G8 antibody treated group. These results show that the 1G8 monoclonal antibody can inhibit the growth of prostate tumors when used alone. As with the studies described supra, there was no apparent toxicity associated with the treatment of these animals with the 1G8 mAb.

The effect of the 1G8 monoclonal antibody on the growth of prostate cancers generated with PC-3 cells was also determined. PC-3 cells do not express PSCA. As shown in FIG. 65, the 1G8 antibody had no effect on the development of PC-3 xenograft tumors, in sharp contrast to its effect on PSCA-expressing LAPC-9 xenografts. These results clearly show that the 1G8 antibody is inhibiting tumor cell growth through the PSCA antigen.

C2: PSCA mAbs 2A2 and 2H9

Two anti-PSCA monoclonal antibodies of different isotypes were evaluated simultaneously for prostate tumor growth inhibition in vivo. Anti-PSCA mAbs 2A2 (IgG2a isotype) and 2H9 (IgG1 isotype) were tested for prostate tumor inhibition as described in Example 18-C1, immediately above. The results presented in FIG. 55 demonstrate striking inhibition of tumor cell growth in the anti-PSCA mAb treated groups versus the control groups. Specifically, the average tumor volume at the final time point was 483 mm^3 for mice treated with murine IgG (day 42), 49 mm^3 for mice treated with the 2A2 mAb (day 42), and 72 mm^3 for the mice treated with 2H9 mAb (day 42). More significantly, tumor incidence was 6/6 mice in the mouse IgG control group, versus 2/7 for the 2A2-treated group and 1/7 for the 2H9-treated group. In the 2A2 treated group, the first tumor appeared at day 25 and the second tumor at day 42. In the

2H9 treated group the single tumor present appeared at day 21. In the mouse IgG control group, 4/6 of the mice had developed tumors by day 21. As with the in vivo studies described above, there was no apparent toxicity associated with the treatment of these animals with the 2A2 or 2H9 mAbs.

PSA levels in the serum of the treated mice were significantly lower than in control mice, and correlated directly with tumor volume (FIG. 56). At week 6, the mean PSA serum level in the mouse IgG control group was 35 ng/ml, 2 ng/ml in the 2A2 group, and 8 ng/ml in the 2H9 group.

This study further supports the conclusion that a single "naked" anti-PSCA monoclonal antibody is sufficient for anti-tumor activity. In addition, these data demonstrate that mAbs recognizing different PSCA epitopes are effective, and that the anti-tumor effect is not dependent upon a single IgG isotype since both IgG1 (1G8, 2H9) and IgG2a (2A2) mAbs inhibited tumor growth.

C3: PSCA mAbs exert growth inhibitory effect specifically through PSCA

In order to demonstrate that PSCA mAbs exert tumor growth inhibition specifically through the PSCA protein, a tumor inhibition study with the 1G8 mAb and PC-3 tumor xenografts was conducted. PC-3 cells do not express endogenous PSCA. This study was conducted as described in Section C1 of this Example, above. The results, shown in FIG. 65, show that the PSCA mAb 1G8 had no effect on the growth of PC-3 tumors in mice over a 40 day period. The results are shown, for comparison, together with a parallel study of the effect of 1G8 on LAPC-9 prostate tumor xenografts (Example C1, above).

C4: PSCA mAb 3C5 inhibits the growth of established LAPC-9 prostate tumors in vivo

In order to determine whether PSCA mAbs could effect growth of established tumors, the following study was conducted. Briefly, a cohort of SCID mice were injected with 10^6

LAPC-9 cells SQ, essentially as described in examples C1 and C2. After tumors reached a size of approximately 100 cubic millimeters, mice were segregated into two groups, a control group receiving mouse IgG and a treatment group receiving PSCA mAb 3C5. Each mouse was injected IP with control IgG or 3C5 mAb according to the following protocol: 1 mg per injection, three times per week for the first 2 weeks, followed by two times per week in the third week. Tumor volume and PSA measurements were determined as above. The results, shown in FIG. 57, indicate that the 3C5 mAb inhibits the growth of established LAPC-9 prostate tumors in vivo. In at least some of the treatment group mice, tumor regression up to 50% of the initial, pre-treatment size of the tumor was observed.

Example 19:

In vitro assays for characterizing PSCA monoclonal antibodies

19-A: Antibody-dependent cell cytotoxicity assay

To determine if the anti-PSCA mAbs sensitize tumor cells to ADCC, the following assay is performed. First, for NK cell mediated ADCC, spleen cells from SCID mice are cultured for 5 days in vitro as described by Hooijberg et al., 1995, Cancer Res. 55: 2627-2634. The activated cells are then co-cultured with ^{51}Cr-labeled LAPC-9, LNCaP-PSCA, or LNCaP target cells for four hours in the presence of either anti-PSCA mAbs or a control mouse IgG. LNCaP serves as a negative control in all assays since it does not express PSCA. If single mAbs are used, the respective mouse IgG isotype control is also used. NK activity of the activated spleen cells is determined by incubation with the murine NK-sensitive target YAC-1. In all cases, killing is determined by ^{51}Cr-release into the medium. Spontaneous release is determined after incubation of labeled cells only, and total release by incubation of labeled cells with 5% Triton X-100. The percent of specific cell lysis is determined by:

$$\% \text{ Cell Lysis} = \frac{\text{Experimental } ^{51}\text{Cr release - spontaneous } ^{51}\text{Cr release}}{\text{Total } ^{51}\text{Cr release - spontaneous } ^{51}\text{Cr release}}$$

19-B: Antibody-dependent macrophage-mediated cytotoxicity assay

To determine whether the anti-PSCA mAbs sensitize tumor cells to ADMMC, the following assay is performed. Peritoneal macrophages are activated by intraperitoneal injection of SCID mice with Brewer's thioglycollate medium as described by Larson et al., 1988, Int. J. Cancer 42: 877-882. After four days, cells are collected by intraperitoneal lavage, and the percent of activated macrophages determined by Mac-1 staining. For the assay, the activated macrophages are co-cultured with ^{3}H-thymidine labeled LAPC-9, LNCaP-PSCA, and LNCaP target cells for 48 hours in the presence of either anti-PSCA mAbs or control mouse IgG. At the end of the assay, supernatants are harvested from the wells and killing is determined by the amount of ^{3}H-thymidine released as described above for ^{51}Cr release.

19-C: Complement-mediated tumor cell lysis assay

Destruction of tumor targets by complement-dependent lysis may be performed according to the method described by Huang et al., 1995, Cancer Res. 55: 610-616. For example, LAPC-9, LNCaP-PSCA, and LNCaP cells are labeled with ^{51}Cr and then incubated on ice for 30' with either anti-PSCA mAbs or a mouse IgG control. After washing to remove unbound antibody, the cells are incubated with rabbit complement at 37°C for 2 hr, and cell lysis measured by ^{51}Cr-release into the supernatant. The percent cell lysis will be determined as described above.

19-D: Cell proliferation assay

The effect of anti-PSCA mAbs on cell proliferation may be determined by an MTT assay. Briefly, LNCaP-PSCA or LNCaP cells are cultured for 72 hr with varying amounts of

either anti-PSCA mAbs or mouse IgG as a control. At the end of the incubation period, the cells are washed and incubated in a solution of MTT for 4 hr. Proliferation is indicated by dehydrogenase mediated conversion of the MTT solution to a purple color and measured at a wavelength of 570 nm.

19-E: Assay for colony formation in soft agar

Colony formation in the presence of anti-PSCA mAbs may be measured by growth of cells in soft agar. Briefly, 1 x 10^4 LNCaP-PSCA or LNCaP cells are plated in medium containing Nobel agar. A dilution series of anti-PSCA mAbs is then added to plates in duplicate to determine the effect on colony growth. Mouse IgG is used as a control. Macroscopic colonies are counted after 14-21 days in culture.

Example 20:

PSCA capture ELISA

A PSCA capture ELISA was developed in order to measure PSCA levels in serum prior to treatment with anti-PSCA mAbs and provides information useful in determining the therapeutic dosage regimen. The assay may also be useful in monitoring patient response to the therapy.

A schematic representation of the assay format is shown in FIG. 50B. Briefly, affinity purified anti-PSCA peptide sheep polyclonal antibody (directed against amino acids 67-81 of the PSCA protein) and anti-PSCA monoclonal antibody 1G8 are used as capture antibodies and are coated microtiter wells. After coating, incubation with a dilution series of test antigen is conducted in order to generate a standard curve. Patient serum is added to the wells and incubated at room temperature. After incubation, unbound antibody is washed with PBS. Anti-PSCA monoclonal antibodies 2A2, 3C5 and 4A10 (IgG2a isotype), which recognize different epitopes on the PSCA protein, are used as detection antibodies, and are added to the wells, incubated, and the wells washed to remove

unbound antibody. The captured reaction is then visualized by the addition of an anti-mouse Ig2a-horseradish peroxidase-conjugated secondary antibody followed by development with 3,3' 5,5' tetramethylbenzidine base substrate and OD determinations taken.

A schematic representation of the standardization and control antigens are shown in FIG. 50A. Briefly, a GST-fusion protein encoding amino acids 18-98 of PSCA is used for generating a standard curve for quantification of unknown samples. A secreted recombinant mammalian expressed form of PSCA is used for quality control of the ELISA assay. This protein contains an Ig leader sequence to direct secretion of the recombinant protein and MYC and 6XHIS epitope tags for affinity purification.

Quantification of recombinant PSCA secreted from 293T cells engineered to express and secrete PSCA is shown in FIG. 51.

Example 21:

Sequence of PSCA mAb genes

The nucleotide sequences of the genes encoding the heavy chain variable regions of murine monoclonal antibodies 1G8, 4A10 and 2H9 were determined using the methods described in Coloma et al., 1992, J Immunol. Methods 153: 89-104. Primers for heavy chain variable region sequencing of mAbs 1G8 and 4A10 were as follows:

HLEAD.1: ggc gat atc cac cat ggR atg Sag ctg Kgt Mat Sct ctt (SEQ ID NO. 25)

CH3': agg gaa ttc aYc tcc aca cac agg RRc cag tgg ata gac (SEQ ID NO. 26)

Primers for heavy chain variable region sequencing of mAb 2H9 were as follows:

HLEAD.2: ggg gat atc cac cat gRa ctt cgg gYt gag ctK ggt ttt (SEQ ID NO. 27)

CH3': agg gaa ttc aYc tcc aca cac agg RRc cag tgg ata gac (SEQ ID NO. 26)

Total RNA was isolated from 1G8, 2H9, and 4A10 hybridoma cells using the Trizol Reagent (Gibco-BRL cat#15596). First strand synthesis reactions on 5 μg of RNA were generated using the Gibco-BRL Superscript II reverse transcriptase reaction according to manufacturers protocols and CH3'. Two μl of the 30 μl first strand reaction was used in the PCR to amplify the variable regions.

First strand cDNA was synthesized from hybridoma RNA using a primer from the constant region of the heavy chain (CH3'). The variable region was amplified using CH3' and a primer designed to the leader sequence (HLEAD.1 and HLEAD.2). The resulting PCR product is sequenced and the complementarity determining regions (CDRs) are determined using the Kabat rules. The nucleotide (SEQ ID NOS:10, 12, and 14) and amino acid (SEQ ID NOS:11, 13, and 15) sequences are shown in FIGS. 58, 59 and 60. An amino acid alignment of the CDRs of these three mAbs is shown in FIG. 61.

Example 22:

PSCA mAb binding affinity

The affinity of PSCA monoclonal antibody 1G8 (described above) was determined using BIAcore™ instrumentation (Uppsala, Sweden), which uses surface plasmon resonance (SPR, Welford K. 1991, Opt. Quant. Elect. 23:1; Morton and Myszka, 1998, Methods in Enzymology 295: 268) to monitor biomolecular interactions in real time. On the basis of general procedures outlined by the manufacturer (Pharmacia), kinetic analysis of the antibody was performed using a recombinant PSCA immobilized onto the sensor surface at a low density (30 RU). Recombinant PSCA was generated as follows. 293T cells transiently transfected or 293 cells stably expressing a CMV-driven expression vector encoding PSCA with a C-terminal 6XHis and MYC tag (pcDNA3.1/mycHIS, Invitrogen) served as source of secreted soluble PSCA protein for purification. The HIS-tagged

PSCA protein was purified over a nickel column using standard techniques. The association and dissociation rates were determined using the software provided by the manufacturer. The results, tabulated below (Table 5), show that 1G8 has a 1 nanomolar K_D, indicating a strong affinity for the PSCA antigen.

TABLE 5
BIOCORE AFFINITY DETERMINATION OF PSCA mAb 1G8

	mAb in solution		
	k_a ($M^{-1}s^{-1}$)	k_d ($M^{-1}s^{-1}$)	K_D (nM)
	1.68×10^5	1.69×10^{-4}	1.0

The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any which are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

Example 23:

Immunohistochemical analysis

This example describes immunohistochemical (IHC) analysis of various formalin-fixed, paraffin-embedded tissues with the seven anti-PSCA mAbs described supra.

MATERIALS AND METHODS

Each of the seven anti-PSCA monoclonal antibodies was tested against: (1) a cell pellet consisting of LNCaP overexpressing PSCA, LNCaP parental, and 293T cells; (2) LAPC9AD xenograft; and (3) benign prostatic hyperplasia (BPH), prostate carcinoma, and normal prostate tissues. All tissue staining was performed by QualTek Molecular Laboratories, Inc (Santa Barbara, CA). Tissue blocks were sectioned at 4 microns and placed onto positively charged Capillary Gap microscope slides (Ventana Medical Systems, Inc., Tucson, AZ). After dewaxing in xylene, followed by hydration through alcohol series, tissue sections were pretreated in a steamer for 20 minutes in the presence of sodium citrate (10 mM, pH 6.0) in order to optimize antibody reactivity.

After cooling for 5 minutes, the slides were immunostained using an ABC-peroxidase technique. Briefly, slides were incubated in blocking serum (normal goat) for 5 minutes, followed by 2 μg/ml anti-PSCA monoclonal primary antibody or 2μg/ml mouse IgG for the negative control (25 minutes), biotinylated secondary antibody-goat-anti-mouse IgG (25 min) and avidin-biotin complex (ABC) conjugated to peroxidase enzyme, Vector Labs, Burlingame, CA (25 minutes). Between the incubation steps, sections were rinsed in buffer. DAB - Diaminobenzidine chromogen (QualTek Molecular Labs) was used to develop the reaction – yielding a brown precipitate. Slides were subsequently counterstained with hematoxylin and then coverslipped. Staining was performed on a TechMate 1000 automated staining instrument (Ventana Medical Systems, Inc., Tucson, AZ) at room temperature.

RESULTS

FIG. 52 shows the IHC results for the anti-PSCA monoclonal antibody 3C5 in the cell pellet, LAPC9AD xenograft, a BPH sample, and a prostate carcinoma tissue (left panel). The cell pellet mix contains three cell types of which only one, the LNCaP-PSCA cells, are expected to stain with anti-PSCA monoclonal. As expected, 3C5 stains strongly approximately 1/3 of the cells. This staining conveniently shows positive and negatively staining cell types on the same slide. The LAPC9AD xenograft is very strongly stained with 3C5 antibodies. Basal and epithelia cells in the ducts of the BPH tissue stain well

but the basal cells are especially prominent. Finally, the prostate carcinoma tissue shows strong staining in the neoplastic ducts. The panel on the right represents the background control consisting of pre-immune mouse serum. No background staining was detectable in any of the samples evaluated.

Example 24:

Inhibition of established LAPC-9 tumor growth and prolonged survival following anti-PSCA antibody treatment. The LAPC-9 xenograft was generated from a bone tumor biopsy of a patient with hormone-refractory metastatic prostate cancer.

The following examples demonstrate that anti-PSCA monoclonal antibodies, 1G8 and 3C5, inhibit the growth of established orthotopic, LAPC-9 prostate tumors, in SCID mice and significantly prolonged their survival. The growth of the tumors was monitored by tracking the level of serum PSA.

Orthotopic Injections

A single cell suspension of LAPC-9 tumors was prepared according to the methods described in Example 18-A. The cell suspension was mixed, at a 1:1 dilution, with Matrigel. The cell suspension was kept on ice prior to the orthotopic injections. The orthotopic injections were performed on male IcR-SCID mice, under anesthesia, using ketamine/xylazine. The anesthetized mice were shaved in the lower abdomen, a 5-8 mm incision was made just above the penis to expose the abdominal wall and muscles. An incision was made through the abdominal muscles to expose the bladder and seminal vescicles, which were then delivered through the incision to expose the dorsal prostate. The LAPC-9 cell suspension was injected into each dorsal lobe using a 500 µl Hamilton syringe. Each lobe was injected with 10 µl of cell suspension corresponding to about 5 x 10^5 cells. After the injections, both incisions were closed using a running suture and the mice were kept under a heat lamp to recover. After the injections, the serum level of PSA was monitored. The mice were treated with a different regimen of 1G8 or 3C5

antibody, depending on the serum level of PSA. After the antibody treatments, the mice were kept alive, to determine the PSA levels as a measure of the tumor growth, and to determine the survival of the mice in each treatment group.

Monitoring the serum levels of PSA:

The level of serum PSA was used to track the tumor size. The mice were bled on an approximate weekly basis, to monitor the levels of serum PSA, which were measured using a commercially-available PSA kit (American Qualex, San Clemente, CA). The mice were segregated into two treatment groups, based on the levels of serum PSA. For example, the groups included: low levels of PSA (e.g., 2-3 ng/ml; FIG. 66 A); and moderate levels of PSA (e.g., 64-78 ng/ml; FIG. 66 B). The serum PSA levels were monitored until the tumors were visible through the abdomen.

The serum PSA levels were monitored on days +9, +15, +22, +30, +37, +44, and +51, relative to the day of injection of the tumor cells (FIG. 66 A). Similarly, the serum PSA levels were monitored on days +13, +21, +27, and +34 (FIG. 66 B), on days +9, +16, +22, +29, and +36 (FIG. 68 A), and on days +7, +14, +21, and +28 (FIG. 68 B).

Treatment with 1G8:

Orthotopic, tumor-bearing mice were established according to the methods described above. Two groups of animals, exhibiting (i) low levels of serum PSA (2-3 ng/ml), or (ii) moderate levels of serum PSA (64-78 ng/ml) were selected for treatment.

The mice having low levels of PSA (e.g., 2-3 ng/ml) were treated with intraperitoneal injection of 2 mg of 1G8 antibody, three times per week for one week, followed by 1 mg of 1G8 three times per week for the next two weeks, followed by 1 mg of 1G8 once per week for the next three weeks (as indicated by the arrows in FIG. 66 A).

The mice having moderate levels of PSA (e.g., 64-78 ng/ml) were treated with intraperitoneal injection of 1 mg of 1G8 antibody, three times per week for three consecutive weeks, followed by a single injection of 1 mg of 1G8 the following week (as indicated by the arrows in FIG. 66 B).

The control mice, having low or moderate levels of PSA, were treated with about 0.5 to 0.8 ml of phosphate buffer solution (Gibco) (FIG. 66 A and B).

Treatment with 3C5:

Similar treatments were performed on orthotopic tumor-bearing mice, using the 3C5 antibody. In the first experiment, 1 mg of 3C5 antibody was administered intraperitoneally three times per week for three consecutive weeks, followed by a single injection of 1 mg of 3C5 (FIG. 68 A). In the second experiment, 2 mg of 3C5 was administered three times per week for the first week, followed by 1 mg three times per week for the next two weeks (FIG. 68 B). The injections were administered on the days indicated by the arrows in FIG. 68 A and B.

Results – Treatment with 1G8 Results in Inhibition of Tumor Growth and Increased Survival:

The serum PSA levels of the tumor-bearing mice were used to track the growth of the tumors, since the serum PSA levels correlated well with the tumor size. The mice bearing LAPC-9 AD tumors, treated with the anti-PSCA monoclonal antibody, 1G8, exhibited a reduction in the rate of increase in serum PSA levels (FIG. 66 A and B). This result indicates that 1G8 inhibits growth of tumors expressing PSCA.

In FIG. 66 A, each data point represents the mean PSA level for mice receiving PBS (n = 6) or 1G8 (n = 6). The mice were bled on the days indicated on the X-axis for PSA determinations.

As shown in FIG. 66 B, the mice were bled on the days indicated on the X-axis for PSA determinations. Each data point represents the mean PSA level for mice receiving PBS (n = 4) or 1G8 (n = 5). In FIG. 67, 4 mice in the PBS-treated group and 5 mice in the 1G8-treated group.

Additionally, the mice having lower serum PSA levels that were treated with the 1G8 antibody exhibited a reduced rate of increase in the level of serum PSA, compared to the mice having higher serum PSA levels that were treated with the 1G8 antibody (e.g., compare the data described by (■) in FIG. 66 A and B). This result suggests that the 1G8 antibody was more effective at reducing tumor growth, when there was a smaller tumor burden, under the administration protocol of this study.

The affect of the 1G8 treatment on survival of the tumor-bearing mice was also monitored. In general, the mice treated with only PBS began to die within 5-6 weeks post-injection, due to local tumor growth and metastasis. In contrast, the mice treated with 1G8 antibody exhibited a prolonged life. For example, the effect on survival was more dramatic in the mice having low serum PSA levels (FIG. 67 A), where the median survival time in the PBS-treated mice was 56.5 days (range 42-71 days) and the median survival time in the 1G8-treated mice was 89 days (range 77-101). In the mice having moderate serum PSA levels (FIG. 67 B) the median survival time in PBS-treated mice was 40 days (range 32-48 days) compared to a median survival time of 78.5 days (range 52-105 days) in the 1G8-treated mice. This indicates an increase of median survival time of 38.5 days in 1G8-treated mice.

The inhibition of tumor growth correlated with prolonged life. Collectively, these results demonstrate that 1G8 treatment inhibited tumor growth and increased the survival time of orthotopic tumor-bearing mice. Thus, these results suggest that treatment with 1G8 increased survival time, by inhibiting tumor growth.

Treatment with 1G8 effectively inhibited tumor growth on smaller orthotopic tumors. Thus, 1G8 may represent an effective therapeutic treatment for minimal residual disease, in recurrent local disease, after primary treatment and in metastatic tumor formation.

The results of mice treated with the 3C5 antibody are similar to the data obtained from mice treated with the 1G8 antibody. The mice bearing LAPC-9 AD tumors, treated with the anti-PSCA monoclonal antibody, 3C5, exhibited a decrease in serum PSA levels. This result indicates that 3C5 inhibits growth of tumors expressing PSCA.

Two separate experiments were conducted to evaluate the effect of 3C5 treatment. The mice treated with 3C5 antibody exhibited a lower rate of increase in the level of serum PSA, compared to the mice treated with phosphate buffer solution (FIG. 68 A and B). This result suggests that the 3C5 antibody inhibited tumor growth.

In FIG. 68 A, each data point represents the mean PSA level for mice receiving PBS (n = 4) or 3C5 (n = 5). In FIG. 68 B, each data point represents the mean PSA level for mice receiving PBS (n = 6) or 3C5 (n = 6).

The mice treated with 3C5 antibody exhibited prolonged life (FIG. 69 A), compared to the mice treated with PBS (FIG. 69 B). In the first experiment, the median survival time of the PBS-treated mice was 52 days (range 45-59 days), compared to more than 92 days (range 59 to +125 days) in the 3C5-treated group (FIG. 69 A) (one mouse is still alive). In the second experiment, the median survival time in PBS-treated mice was 43 days (range 29-57), compared to 57.5 days in the 3C5-treated mice (range 33-82 days) (FIG. 69 B).

The inhibition of tumor growth correlated with prolonged life. Collectively, these results demonstrate that 3C5 treatment inhibited tumor growth and thereby increased the survival time of orthotopic tumor-bearing mice. Thus, these results indicate that treatment with 3C5 increased survival time, by inhibiting tumor growth.

Example 25:

Inhibition of established PC3-PSCA tumor growth and prolonged survival following anti-PSCA antibody treatment alone or in combination with doxorubicin.

The following examples demonstrate that 1G8, an anti-PSCA monoclonal antibody, inhibited the growth of established subcutaneous, PC3-PSCA prostate tumors (AI), growing in SCID mice. Additionally, 1G8, when administered alone or in combination with doxorubicin, inhibited the growth of prostate tumors. Furthermore, treatment with 1G8 prolonged the survival of these mice, when administered alone or in combination with doxorubicin. Treatment with 1G8 and doxorubicin appears to result in a synergistic inhibitory effect on tumor growth and survival.

PSCA-expressing PC3 cells:

PC3-PSCA cells were derived by retroviral gene transfer. Briefly, PSCA cDNA was inserted into the retroviral vector pSR-α (Muller, et al., 1991 *Molec. Cell. Biol.* 11:1785-1792)). Amphotropic retrovirus was generated by co-transfection of 293T cells with pSR-α containing PSCA and a retroviral helper plasmid containing the amphotropic envelope protein. PC3 cells were subsequently infected with the PSCA containing amphotropic retrovirus, and 48 hours after infection the cells were selected by growth in medium containing the neomycin analogue G418. After 2-3 weeks of selection and expansion, a Western blot was performed to confirm that the PC3-PSCA cells express PSCA protein. Parental PC3 or PC3 cells infected with an empty vector that did not contain PSCA were both negative for PSCA protein expression.

Subcutaneous Injections:

PC3-PSCA cells were grown in T-150 flasks in DMEM + 10% FBS prior to the injections. The cells were grown to log phase, harvested, washed, counted, then mixed with Matrigel at a 1:1 dilution, and kept on ice. For injection, male IcR-SCID mice were

shaved on their flanks, and each mouse received a single subcutaneous injection of about 1 x 10^6 cells in a volume of 100 µl on the right flank. The growth of PC3-PSCA tumors was followed by caliper measurements of the growing tumors. The mice were selected for treatment when the tumor reached the size of 100-200 mm^3, at approximately 9 days after the subcutaneous injection. The tumor size was measured at days +9, +15, +23,+29, +36, and +43 post injection.

Treatment with PBS:

The control mice were treated with about 0.5 to 0.8 ml of phosphate buffer solution (Gibco) (FIG. 66 A and B).

Treatment with 1G8:

The mice treated with 1G8, were administered 1 mg of 1G8, three times per week for three consecutive weeks.

Treatment with Doxorubicin:

An LD_{50} experiment was performed to determine the maximum tolerable dose of doxorubicin. Doxorubicin (Sigma) and was resuspended in sterile PBS. Doxorubicin was administered by intraperitoneal injection, at the following doses: 50 µg, 25 µg, 12.5 µg, and 6.75 µg. At the highest dose, 50 µg, all the mice died within 2 weeks. At the lower dose ranges, the mice survived for more than 4 weeks. The maximal tolerable dose was 25 µg.

The mice treated with only doxorubicin, were administered 25 µg, once weekly for three consecutive weeks.

Treatment with 1G8 and doxorubicin:

The mice treated with 1G8 and doxorubicin, were administered 1 mg of 1G8 three times per week for three consecutive weeks (FIG. 70; 1G8 = arrows), and were administered 25 μg of doxorubicin once weekly for three consecutive weeks (FIG. 70; doxorubicin = (●)).

Results - Treatment with 1G8 alone or in Combination with Doxorubicin Results in Inhibition of Tumor Growth:

The mice bearing PC3-PSCA tumors, treated with anti-PSCA monoclonal antibody, 1G8, alone or in combination with doxorubicin, exhibited a decrease in tumor growth compared to mice treated with phosphate buffer solution or doxorubicin alone (FIG. 70). These results indicate that 1G8 inhibits the growth of tumors expressing PSCA. These results also suggest that the combination of 1G8 and doxorubicin act synergistically to inhibit tumor growth.

Each data point represents the mean tumor volume for mice receiving PBS (n = 5), doxorubicin (n = 6), 1G8 (n = 6), or 1G8 + doxorubicin (n = 6).

The mice treated with doxorubicin exhibited a slightly lower tumor growth rate, compared to mice treated with PBS (e.g., 4 % growth inhibition at day 43). In contrast, mice treated with 1G8 antibody alone exhibited a greater reduction in tumor growth rate, compared to the mice treated with PBS (e.g., 20% growth inhibition at day 43). The mice treated with the combination of 1G8 and doxorubicin exhibited a slightly greater reduction in tumor growth rate, compared to the mice treated with PBS (e.g., 36% growth inhibition at day 43).

Treatment with 1G8 alone, in this subcutaneous model using PC3-PSCA xenografts, effectively inhibited tumor growth on established androgen-independent tumors (e.g., PC3 cells are hormone refractory). Furthermore, the combination of 1G8 and doxorubicin showed augmented tumor growth inhibitory effects. Thus, treatment with the combination of 1G8 and doxorubicin represents an effective therapeutic treatment for

prostate cancer patients with metastatic disease who have failed hormone ablation therapy.

Example 26:

Anti-PSCA Monoclonal Antibodies Circulate and Target PSCA-Expressing Tumors

In one study, SCID mice bearing established, subcutaneous, LAPC-9 AD tumors, described in Example 18 C4 above, were treated with either control mouse IgG or 3C5 anti-PSCA mAb as described. On day 34, 6 days after the last antibody treatment, the mice were sacrificed and the tumors were explanted. In a different study, tumor-bearing SCID mice were treated with control mouse IgG or 1G8. The presence of antibody in the tumor samples from both studies was detected by either Western blot analysis or immunohistochemistry (IHC).

Immunohistochemistry:

Explanted tumors were fixed in formalin and embedded in paraffin for immunohistochemical analysis (performed by QualTek Molecular Labs, Santa Barbara, CA). The paraffin blocks were sliced, the slices were fixed on slides, and the slides were probed with biotinylated goat anti-mouse IgG followed by an avidin-biotin complex (ABC) conjugated to peroxidase enzyme (Vector Labs, Burlingame, CA). DAB (diaminobenzidine) chromogen was used to develop the reaction which yielded a brown precipitate. Slides were subsequently counterstained with hematoxylin and then coverslipped. Staining was performed on a TechMate 1000 automated staining instrument (Ventana Medical Systems, Inc., Tucson, AZ) at room temperature (FIG. 71).

Results – Immunohistochemistry:

FIG. 71 demonstrates that the 3C5 antibody localized in the LAPC-9 AD tumors from the 3C5-treated mice, but not with control IgG- treated mice. Additionally, antibody could be detected throughout the tumor.

Western Blotting:

Explanted tumors from 3 mice in the IgG-treated group and the 3C5-treated group, (e.g., the mice as described in Example 18 C4 above), were lysed in boiling SDS sample buffer. The cell lysates were electrophoresed in SDS-PAGE gels, transferred to nitrocellulose filters, probed with goat anti-mouse IgG-HRP antibodies (Southern Biotech, Birmingham, AL), and visualized by enhanced chemiluminescence. The mouse IgG control antibody and 3C5 were also run on the gel as controls (FIG. 72).

In a similar experiment, LAPC-9 AD subcutaneous tumor-bearing mice were treated with either control mouse IgG or the 1G8 anti-PSCA mAb. On day 30, which was 7 days after the last antibody treatment, the mice were sacrificed and tumors were explanted. Western blot analysis was performed on explanted tumors from 3 mice in each group. The explanted tumors were lysed in boiling SDS sample buffer, the cell lysates were electrophoresed in SDS-PAGE gels, transferred to nitrocellulose, probed with goat anti-mouse IgG-HRP antibodies (Southern Biotech, Birmingham, AL), and visualized by enhanced chemiluminescence. The mouse IgG control antibody and 1G8 were also run on the gel as controls (FIG. 73).

Results – Western Blotting:

FIG. 72 demonstrates that IgG heavy and light chains were readily detected in tumor lysates from the 3C5 treated mice, but not in the mouse IgG control treated mice.

FIG. 73 demonstrates that IgG heavy and light chains were readily detected in tumor lysates from the 1G8 treated mice, but not in the mouse IgG control treated mice.

These results demonstrate that anti-PSCA mAbs, such as 3C5 and 1G8, can circulate and target a PSCA-expressing tumor, after administration to tumor-bearing mice. Furthermore the antibody localization is specific since control mouse IgG, which does not recognize PSCA, is either absent from the tumors, or present at very low levels when

compared to tumors from anti-PSCA treated mice. These results suggest that anti-PSCA mAbs have the potential to circulate through the body and localize to primary and metastatic, PSCA-expressing tumors in cancer patients. Furthermore, conjugated anti-PSCA mAbs may be capable of effectively destroying PSCA-expressing tumors for local, locally recurring and metastatic disease.

What is claimed is:

1. An antibody which specifically binds to PSCA on the surface of carcinoma cells, and is internalized within the carcinoma cells to which it binds.

2. An antibody which specifically binds to PSCA on the surface of carcinoma cells, and is cytotoxic to the carcinoma cells to which it binds.

3. An antibody which specifically binds to PSCA on the surface of carcinoma cells, and is cytostatic to the carcinoma cells to which it binds.

4. An antibody which specifically binds PSCA on the cell surface of carcinoma cells, and is internalized and kills the carcinoma cells to which it reacts.

5. An antibody which specifically binds to PSCA on the surface of carcinoma cells, and is internalized and is cytostatic to the carcinoma cells to which it binds.

6. An antibody, comprising an antigen binding site, wherein the antigen binding site recognizes and binds the N terminal region of PSCA.

7. An antibody, comprising an antigen binding site, wherein the antigen binding site recognizes and binds the C terminal region of PSCA.

8. An antibody, comprising an antigen binding site, wherein the antigen binding site recognizes and binds the middle region of PSCA.

9. The antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8 which is a monoclonal antibody.

10. A monoclonal anti-idiotypic antibody reactive with an idiotype on the antibody of of claim 1, 2, 3, 4, 5, 6, 7 or 8.

11. A recombinant protein which is a murine/human chimeric antibody having (a) a variable region of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8 and (b) a constant region of human origin.

12. A polypeptide that binds PSCA comprising the antigen-binding region of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8.

13. A monoclonal antibody, the antigen-binding region of which competitively inhibits the immunospecific binding of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8 to its target antigen.

14. A bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the antibody of claim 1, 2, 3, 4, 5, 6, 7, or 8 binds.

15. An Fab, F(ab')2 or Fv fragment of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8.

16. A single chain antibody molecule that binds PSCA comprising an antigen binding region of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8.

17. An immunoconjugate comprising the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8 joined to a therapeutic agent.

18. An immunoconjugate comprising the recombinant protein of claim 11 joined to a therapeutic agent.

19. An immunoconjugate comprising the polypeptide of claim 12 joined to a therapeutic agent.

20. An immunoconjugate comprising the monoclonal antibody of claim 9 joined to a therapeutic agent.

21. An immunoconjugate comprising the bispecific antibody of claim 14 joined to a therapeutic agent

22. An immunoconjugate comprising the single chain antibody molecule of claim 16 joined to a therapeutic agent.

23. The immunoconjugate of any one of claims 17-22, wherein the therapeutic agent is a cytotoxic agent.

24. The immunoconjugate of claim 23, wherein the cytotoxic agent is selected from a group consisting of ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, *Pseudomonas* exotoxin (PE) A, PE40, abrin, arbrin A chain, modeccin A chain, alpha-sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, and glucocorticoidricin.

25. A pharmaceutical composition useful in killing human cells expressing the PSCA antigen on the cell surface, comprising a pharmaceutically effective amount of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8 and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition useful in killing human cells expressing the PSCA antigen on the cell surface, comprising a pharmaceutically effective amount of the immunoconjugate of any one of the claims 17-22, and a pharmaceutically acceptable carrier.

27. A method for treating a subject suffering from a malignant disease characterized by cells having the PSCA antigen on the cell surface which comprises administering to the subject an effective amount of an immunoconjugate of any

one of the claims 17-22 such that the immunoconjugate binds the PSCA antigen and kills said cells thereby treating the subject.

28. A method for selectively killing tumor cells expressing PSCA comprising contacting said tumor cells with an amount of the antibody of claim 1, 2, 3, 4, 5, 6, 7 or 8 for a time sufficient to kill said cells.

29. A method for prolonging the life of a subject with a cancer associated with PSCA, comprising administering to the subject a monoclonal antibody which binds to PSCA in an amount effective so as to inhibit the cancer, thereby prolonging the life of the subject.

30. The method of claim 29, wherein said antibody is conjugated to a cytotoxic agent.

31. The method of claim 30, wherein said cytotoxic agent is selected from the group consisting of ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diphteria toxin, *Pseudomonas* exotoxin (PE) A, PE40, abrin, arbrin A chain, modeccin A chain, alpha-sarcin, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, and glucocorticoidricin.

32. The method of claim 30, wherein said cytotoxic agent is a radioactive isotope.

33. The method of claim 32, wherein said radioactive isotope is selected from the group consisting of ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

34. The method of claim 29, wherein said monoclonal antibody is not conjugated to a cytotoxic agent.

35. The method of claim 29, wherein the monoclonal antibody comprises murine antigen binding region residues and human antibody residues.

36. The method of claim 29, wherein the monoclonal antibody is a humanized antibody.

37. The method of claim 29, wherein the monoclonal antibody is a human antibody.

38. The method of claim 29, wherein the cancer is prostate cancer.

39. The method of claim 29, wherein the cancer is metastatic prostate cancer.

40. The method of claim 29, wherein the cancer is bladder cancer.

41. The method of claim 29, wherein the cancer is a metastatic bladder cancer.

42. The method of claim 29, wherein the cancer is a pancreatic cancer.

43. The method of claim 42, wherein the cancer is a metastatic pancreatic cancer.

44. The method of claim 29, further comprising administering to the patient a chemotherapeutic drug.

45. The method of claim 29, further comprising administering to the patient hormone ablation therapy.

46. The method of claim 29, further comprising administering to the patient hormone antagonist therapy.

47. The method of claim 29, further comprising administering radiation therapy to the patient.

48. A method of inhibiting the growth of cancer cells expressing PSCA, comprising administering to a patient a combination of monoclonal antibodies which bind to PSCA in an amount effective so as to inhibit growth of the cancer cells.

49. The method of claim 48, wherein the combination of monoclonal antibodies comprise monoclonal antibodies of at least two different isotypes.

50. The method of claim 48, wherein the combination of monoclonal antibodies comprise monoclonal antibodies with different epitope specificities.

51. The method of claim 48, wherein the combination of monoclonal antibodies comprises monoclonal antibodies 1G8, 2A2, 2H9, 3C5, 3E6, 3G3 and 4A10 produced by the hybridomas designated HB-12612, HB-12613, HB-12614, HB-12616, HB-12618, HB-12615, and HB-12617, respectively, as deposited with the American Type Culture Collection.

52. The method of claim 46, wherein the combination of monoclonal antibodies is selected from the group consisting of mAb 1G8, 2A2, 2H9, 3C5, 3E6, 3G3 and 4A10 produced by the hybridomas designated HB-12612, HB-12613, HB-12614, HB-12616, HB-12618, HB-12615, and HB-12617, respectively, as deposited with the American Type Culture Collection.

METHODS FOR INDUCING AN IMMUNE RESPONSE TO CANCERS EXPRESSING PSCA; ~~PROSTATE STEM CELL ANTIGEN AND USES THEREOF~~

ABSTRACT OF THE DISCLOSURE

The invention provides a novel prostate cell-surface antigen, designated Prostate Stem Cell Antigen (PSCA), which is widely over-expressed across all stages of prostate cancer, including high grade prostatic intraepithelial neoplasia (PIN), androgen-dependent and androgen-independent prostate tumors.